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Involvement of vascular endothelial growth factor, CD44 and CD133 in periodontal disease and diabetes: an immunohistochemical study

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Abstract

Aim: The aim of this study was to investigate the relationship between expression of angiogenic and regeneration markers and periodontal disease in subjects with/without diabetes mellitus.

Material and Methods: Immunohistochemical detection of vascular endothelial growth factor (VEGF), CD44 and CD133 was performed in 16 samples each of (1) healthy gingiva from non-diabetic subjects (controls), (2) gingiva from non-diabetic subjects with periodontitis, (3) gingiva from subjects with type 1 diabetes and periodontitis, (4) gingiva from subjects with type 2 diabetes and periodontitis.

Results: Diseased gingivae from patients with diabetes and periodontitis had greater clinical measures of periodontal disease than those with periodontitis only. VEGF expression was significantly enhanced in epithelial and endothelial cells from patients with periodontitis compared with controls ($p < 0.05$). Epithelial CD44 expression was strong in all groups, while CD44 was significantly enhanced ($p < 0.05$) in connective tissue cells from both diabetic groups. Epithelial and endothelial CD133 expression was comparable in all patients except those with type 2 diabetes and periodontitis, where it was not detected. Stromal CD133 expression was significantly lower in patients with type 2 diabetes and periodontitis and was increased in periodontitis patients ($p < 0.05$).

Conclusions: The involvement and high expression of VEGF, CD44 and CD133 in periodontal disease may predict a greater regeneration capacity of gingival tissue.

Key words: CD133; CD44; diabetes mellitus; periodontal disease; regeneration; VEGF

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Conflict of interest and source of funding statement

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Periodontal disease is characterized by destructive processes that affect the supporting structures of the teeth and involve numerous and complex causes and symptoms. These disorders are due to the interaction of multiple factors, such as exposure to bacteria and viruses, inflammation and genetic factors (Borrell & Papapanou 2005, Loos et al. 2005, Shapira et al. 2005, Tonetti et al. 2005, Van der Velden et al. 2006). In turn, periodontal diseases have powerful, multiple influences on the occurrence and severity of systemic disorders, such as cardiovascular (Nibali et al. 2007) and respiratory conditions and diabetes mellitus (DM) (D'Aiuto et al. 2005).

Because DM is a significant independent risk factor for development of

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gingivitis and periodontitis (Lalla 2007), its association with periodontal disease has been extensively studied for more than 50 years (Mealey 2006). The American Diabetes Association (ADA) has classified DM in two main types on the basis of underlying pathophysiology, pharmacological treatment and aetiology (ADA 2006): type 1 DM, typical of younger patients, defined by hyperglycaemia with an absolute deficiency of insulin secretion (Genuth et al. 2003) and type 2 DM, typical of older patients, resulting from insulin resistance rather than from the absence of insulin production (Stewart et al. 2001). Because periodontal disorders generally affect patients aged 50 years or older, the maintenance of a healthy periodontium may be more difficult in these subjects (Persson 2006).

Several studies have documented the importance of inflammation in periodontal disease, suggesting a major role for diabetes and its complications (Kiran et al. 2005, Mealey 2006).

An accumulation of proinflammatory cytokines and inflammatory cells has been observed in the extravascular periodontal connective tissue of patients with periodontal disease and DM, especially type 2 DM (Yki-Jarvinen et al. 1989, Murakami et al. 1997). Inflammation increases insulin resistance, further impairing glycaemia regulation mechanisms (Yki-Jarvinen et al. 1989). Moreover, hyperglycaemic environments induce non-enzymatic glycosylation of several proteins, including collagen, leading to accumulation of glycation end-products (AGEs). These play a significant role in the complications of diabetes (Martorelli de Lima et al. 2004), by altering the function of several extracellular matrix components, modifying matrix–matrix and cell–matrix interactions (Seibold et al. 1985), and inducing marked changes in endothelial cell function, capillary growth and vessel proliferation (Wautier & Guillausseau 1998).

Vascular endothelial growth factor (VEGF) induces endothelial cell proliferation, stimulates angiogenesis and increases vascular permeability (Unlu et al. 2003). DM may have an inductive effect on periodontal VEGF levels during periodontal disease (Sakallioglu et al. 2007) and VEGF may be an important factor in the onset of gingivitis and in its progression to periodontitis (Giannobile et al. 2003).

CD44 is an integral membrane glycoprotein involved in tooth cell differentiation, inflammatory cell accumulation and in both cell–cell and cell–matrix interactions in the periodontium (Leonardi et al. 2006). CD44 is often bound to hyaluronan (HA); its activation by HA enhances keratinocyte differentiation, which is required for normal horny layer and epidermal barrier function. The CD44-HA complex may also be associated with the inflammatory reaction seen in periodontal disease (Bourguignon et al. 2006).

CD133 was first described as a marker of human haematopoietic stem cells (Yin et al. 1997). Recently, it has been proposed as a universal marker for tissue stem/progenitor cells (Bussolati et al. 2005) and has been shown to be critical for tissue regeneration processes (Ivanovski et al. 2006). Periodontal restoration requires formation of new connective tissue at the root surface, a process that involves periodontal fibres as well as proliferation of new blood vessels. Unfortunately, current regeneration techniques are unreliable, yielding at best only partial regeneration (Zohar & Tenenbaum 2005).

The present immunohistochemical study was devised to assess the expression of VEGF, CD44 and CD133 in periodontal tissue [diseased gingiva (DG)] of diabetic and non-diabetic individuals with periodontitis to gain further insights into the biological mechanisms involved in the relationship between pathogenesis of periodontal disorders and diabetes.

Material and Methods

Participants

Gingival samples were obtained from subjects undergoing surgery to remove an impacted third molar or to treat periodontitis. Donors were non-smokers without systemic complications or a history of allergies who did not have an abnormal platelet count (as demonstrated by a complete blood count 1 month before surgery), had not been treated for periodontitis over the previous 2 years and had taken no antibiotics in the 6 months preceding surgery.

The study was approved by the Ethics Committee of Marche Polytechnic University. All subjects gave their informed consent to participate in the study.

There were 64 gingiva samples:

- *32 samples from non-diabetic patients* without (*n = 16*; CTRL*)* or with periodontitis (*n = 16*; DG*)* were obtained from the Dental Science Institute, Marche Polytechnic University, Ancona, Italy. All had glycosylated haemoglobin (HbA_{1c}) in the non-diabetic range ($<6.1\%$) based on the assay used in the study, and fasting plasma glucose <6.0 mmol/l.
- *32 samples from diabetic patients* with type 1 DM (*n = 16*; DG-1DM) or type 2 DM (*n = 16*; DG-2DM), who were selected from a pool of 283 diabetic subjects followed at ''A. Murri'' Hospital Diabetes Centre, Fermo, Italy. At the time of the study, none of those without periodontitis met the inclusion criteria.

The DM patients had relatively stable glycaemia control, as demonstrated by a difference not exceeding 1% in at least two HbA_{1c} assays over the previous 6 months. Exclusion criteria were any conditions that might affect glucose control or erythrocyte turnover during the study period. Subjects with haemoglobinopathy were also excluded, to avoid any interference with the HbA_{1c} assay (Bry et al. 2001), as were those with macrovascular disease, defined as a history of ischaemic heart disease, stroke, transient ischaemic attack or peripheral vascular disease.

Clinical parameters included body mass index (BMI), diabetes duration, fasting blood glucose, HbA_{1c} and C-reactive protein (CRP). Plasma glucose was assayed on a Hitachi 911 analyser (Hitachi, Tokyo, Japan) using the glucose oxidase method (intra- and inter-batch CV $<4\%$). HbA_{1c} was measured in whole blood using ion-exchange high-performance liquid chromatography with the Bio-Rad Variant Haemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). Plasma high-sensitivity CRP concentrations were measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostic, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled to latex microparticles.

The 32 diabetic and the 16 DG patients had a diagnosis of generalized, chronic, severe periodontitis (Armitage 1999), with more than eight disease sites with clinical attachment level (CAL) >5 mm and radiographic evidence of bone loss.

The clinical periodontal measures were assessed by the same trained examiner (S. D. A.), who was blind to group assignment, using the same type

of periodontal probe (15 mm; University of North Carolina; UNC-15 Hu-Friedy®, Chicago, IL, USA). Outcome measures were (1) plaque index (PI) (Loë 1967); (2) gingival index (GI) (Loë 1967); (3) bleeding on probing (BOP); (4) probing depth (PD), measured to the nearest millimetre from the gingival margin to the bottom of the pocket using a calibrated periodontal probe (probe tip diameter = 0.5 mm) and (5) CAL, measured from the cemento-enamel junction to the bottom of the pocket.

Collection of gingival biopsies

For the extraction of the impacted third molar, an intrasulcular incision was made after local anaesthesia on the buccal or the lingual aspect of the tooth (CAL > 5 mm) with a 15C blade in all the 64 subjects. Two oblique release incisions were made from the mesial and distal extremities of the intrasulcular buccal/lingual incision, and the coronal portion of the gingiva (4 × 5 mm) was excised.

Immunohistochemistry

Gingival biopsies were frozen in liquid nitrogen and stored at −70°C; 6-μm-thick sections were air-dried overnight, fixed in acetone for 10 min. and incubated overnight at 4°C with the following monoclonal antibodies: anti-VEGF (dil. 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-CD44 (dil. 1:50; Vector Laboratories, Burlingame, CA, USA) and anti-CD133 (dil. 1:50; Miltenyi Biotec, Auburn, CA, USA). The reaction was visualized using the streptavidin-biotin-peroxidase technique (Dako-Envision Plus/HRP peroxidase kit; Dako SpA, Milano, Italy). Sections were incubated with 3,3-diaminobenzidine (0.05 diaminobenzidine in 0.05 M Tris buffer, pH 7.6 and 0.01% hydrogen peroxide) (Sigma-Aldrich, Milano, Italy) and counterstained with Mayer's haematoxylin (Bio-Optica SpA, Milano, Italy). Foetal kidney tissue was prepared for immunohistochemistry as positive control as detailed above (Unlu et al. 2003). Negative controls were performed by substituting the primary antibody with non-immune serum. Immunostaining was observed in epithelial and connective tissue cells and in endothelial cells of sub-epithelial connective tissue vessels. Cells positive for VEGF, CD44 and CD133 were counted under a Nikon Eclipse E600 light microscope (Nikon, Düsseldorf, Germany) at 400 × magnification from at least 1,000 cells in the more representative fields, and their number was expressed as a proportion of positive cells.

Antibody immunoreactivity was evaluated by two independent observers (G. L. and A. Z.).

Statistical analysis

Outcome measures were expressed as mean ± standard deviation (SD). Continuous variables for multiple groups were compared by ANOVA. Post hoc analysis was performed with Dunnett's multiple comparison test using non-diabetic patients without periodontal disease as the reference (CTRL). ANOVA was also used to validate the impact of diabetes on periodontal status.

Positivity for VEGF, CD44 and CD133 was expressed as mean ± SD; differences among groups were analysed by ANOVA and Bonferroni's test. Significance was set at $p \leqslant 0.05$.

Results

The clinical characteristics and periodontal measures of all the subjects are shown in Table 1.

Patients with periodontal disease had significantly greater age, CRP, GI, BOP, PD and CAL compared with CTRL subjects. GI and BOP were significantly higher in DG-1DM patients, while age was significantly higher in DG-2DM subjects.

After correction for age, BMI and sex, the two groups of diabetic individuals had significantly greater CRP, GI, BOP and PD compared with patients with periodontal disease, whereas CAL was significantly increased only in the DG-2DM group.

Comparison between DG-1DM and DG-2DM showed significant differences for age, which was greater in DG-2DM patients, and for GI and BOP, which were more elevated in DG-1DM patients.

The immunohistochemical data are shown in Table 2.

VEGF expression

In CTRL samples, faint VEGF expression was detected in gingival epithelium, while endothelial cells showed moderate positivity (Fig. 1a).

VEGF was diffusely expressed throughout the epithelium, mainly in the basal layer, and in endothelial cells from non-diabetic DG (Fig. 1b). It was significantly increased in epithelial and endothelial cells from DG, DG-1DM (Fig. 1c) and DG-2DM (Fig. 1d) compared with CTRL samples ($p < 0.05$). Moreover, a significantly larger number of VEGF-positive cells was observed in the epithelium and vessels of DG-1DM *versus* non-diabetic DG ($p < 0.05$).

CD44 expression

A similar strong CD44 epithelial expression was found in all the groups (Fig. 2a–d).

In contrast, significantly greater ($p < 0.05$) CD44 positivity was observed in connective tissue cells from DG-1DM (Fig. 2c) and DG-2DM (Fig. 2d) compared with CTRL (Fig. 2a) and DG samples (Fig. 2b).

CD133 expression

Epithelial cells showed good CD133 expression that was similar in all the groups (Fig. 3a–d) except DG-2DM (Fig. 3d), where it was not detected. In these individuals, CD133 expression was significantly lower also at the level of connective tissue cells compared with the other groups. Endothelial cells showed moderate CD133 staining in CTRL (Fig. 3a), non-DM (Fig. 3b) and DG-1DM subjects (Fig. 3c), whereas gingiva from the DG-2DM group was negative.

Discussion

More than 3% of the world population suffers from DM, whose prevalence and incidence are rising yearly.

While type 1 DM typically affects younger patients, type 2 DM is an emblematic disorder of the elderly (Meneilly & Tessier 2001, Chau & Edelman 2001), where periodontal tissues exhibit the distinctive features of ageing: reduction of the oral mucosa, transformation of extracellular matrix elastic fibres into collagen fibres with loss of connective tissue elasticity, small-artery arteriosclerosis and gingival hypertrophy caused by periodontal tissue inflammation (Ferrari et al. 2007).

Table 1. Clinical characteristics and periodontal measures of non-diabetic and diabetic patients

	Patients without periodontitis	Patients with periodontitis			*p*-value (DG-1DM *versus* DG-2DM)	*p*-value (all groups)
		non-DM patients	DM patients			
	non-DM patients (CTRL)	non-DM patients (DG)	type 1 DM patients (DG-1DM)	type 2 DM patients (DG-2DM)		
Clinical characteristics						
No. of subjects	16	16	16	16	–	–
M/F	8/8	9/7	7/9	6/10	–	–
Age (years)	34 ± 3.65	47 ± 7.87**	44.75 ± 16.86**	72.25 ± 3.3*	NS	<0.001
BMI (kg/m^2)	24.3 ± 2.9	24.8 ± 3.1	26.4 ± 4.1***	26.7 ± 3.5***	NS	0.01
Duration of diabetes (years)	–	–	24.5 ± 12.7	25.7 ± 8.9	NS	–
Fasting glucose (mmol/l)	4.9 ± 0.7	5.1 ± 0.8	9.2 ± 2.8*	9.0 ± 1.9*	NS	<0.001
HbA_{1c} (%)	5.4 ± 0.6	5.6 ± 0.5	8.7 ± 1.8*	8.5 ± 1.6*	NS	<0.001
CRP (mg/l, range)	0.99(0.52–1.97)	1.42(0.63–2.36)***	1.92(0.74–4.23)**	2.17(0.89–4.58)**	NS	<0.01
Periodontal measures						
Plaque index	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.5	NS	NS
Gingival index	0.4 ± 0.3	1.8 ± 0.5***	2.8 ± 0.9**	1.9 ± 0.7***	<0.01	<0.01
Bleeding on probing	0.1 ± 0.6	1.6 ± 0.6***	2.6 ± 0.6**	1.7 ± 0.8***	<0.01	<0.01
Probing depth	2.1 ± 0.6	6.1 ± 0.8*	7.4 ± 1.9*	6.8 ± 1.9*	NS	<0.001
Clinical attachment level	1.3 ± 0.8	5.6 ± 1.2*	6.3 ± 1.5*	6.5 ± 1.7*	NS	<0.001

Values are mean ± SD.
*$p<0.001$ *versus* CTRL.
**$p<0.01$ *versus* CTLR.
***$p<0.05$ *versus* CTRL.
DG, diseased gingiva; DM, diabetes mellitus; BMI, body mass index; CRP, C-reactive protein.
Post hoc analysis: all groups *versus* CTRL.

Table 2. VEGF, CD44 and CD133 expression differences in healthy *versus* diseased gingivae (DG) of patients with and without DM

	VEGF		CD44		CD133		
	epithelial cells (% ± SD)	endothelial cells (% ± SD)	epithelial cells (% ± SD)	connective tissue cells (% ± SD)	epithelial cells (% ± SD)	connective tissue cells (% ± SD)	endothelial cells (% ± SD)
Non-DM patients							
Patients without periodontitis (CTRL)	10 ± 2.6	25 ± 10.15	85 ± 2.5	30 ± 7.5	65 ± 2.2	20 ± 5.3	20 ± 10
Patients with periodontitis (DG)	40 ± 14.14	40 ± 7.07	85 ± 14.14	35 ± 10	60.5 ± 13.4	39 ± 4.24	20 ± 5
DM patients							
DG patients with type 1 DM (DG-1DM)	70 ± 26.14	50 ± 10	90 ± 8.8	60 ± 6	66.2 ± 7.5	20 ± 14.14	19.5 ± 6.1
DG patients with type 2 DM (DG-2DM)	55 ± 26.46	35 ± 4	87.5 ± 5	65 ± 21	Negative	5 ± 10	Negative
ANOVA and Bonferroni's test $p<0.05$	Epith. cells	Endoth. cells		Conn. tissue cells	Epith. cells	Conn. tissue cells	Endoth. cells
	DG-1DM *versus* CTRL	DG-1DM *versus* DG-2DM		DG-2DM *versus* CTRL	DG-1DM *versus* DG-2DM	DG *versus* DG-2DM	CTRL *versus* DG-1DM
	DG-2DM *versus* CTRL	DG-1DM *versus* CTRL		DG-2DM *versus* DG	CTRL *versus* DG-2DM	DG *versus* DG-1DM	DG *versus* DG-1DM
	DG *versus* CTRL	DG-1DM *versus* DG		DG-1DM *versus* CTRL	DG *versus* DG-1DM	DG *versus* CTRL	DG-1DM *versus* DG-2DM
	DG-1DM *versus* DG	DG *versus* CTRL		DG-1DM *versus* DG		CTRL *versus* DG-2DM	
		DG-2DM *versus* CTRL				DG-1DM *versus* DG-2DM	

VEGF, Vascular endothelial growth factor; DM, Diabetes mellilus.

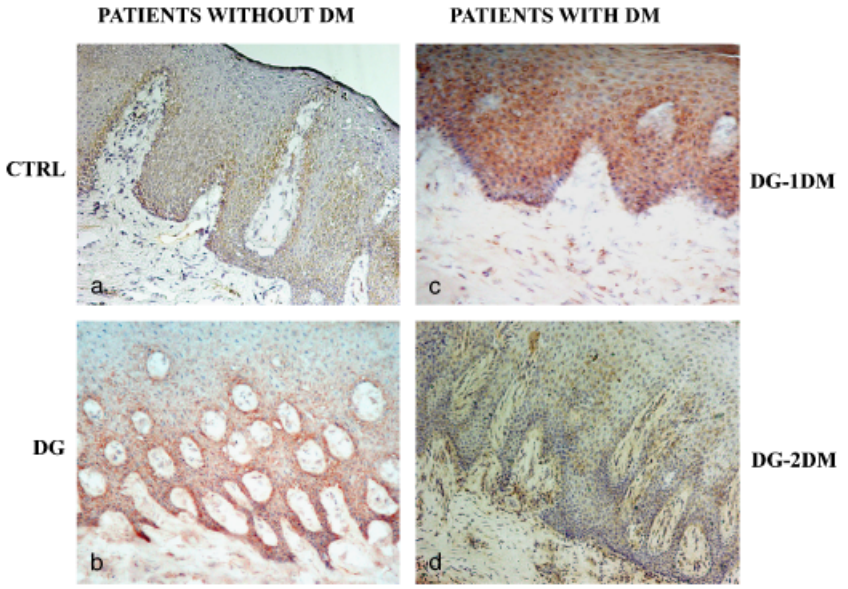

Fig. 1. Immunohistochemical localization of vascular endothelial growth factor in (a) healthy gingiva (CTRL) (immunoperoxidase; 150 × original magnification), (b) diseased gingival (DG) of patients without diabetes mellitus (DM) (immunoperoxidase; 150 × original magnification), (c) DG of patients with type 1 DM (immunoperoxidase; 250 × original magnification), (d) DG of patients with type 2 DM (immunoperoxidase; 150 × original magnification).

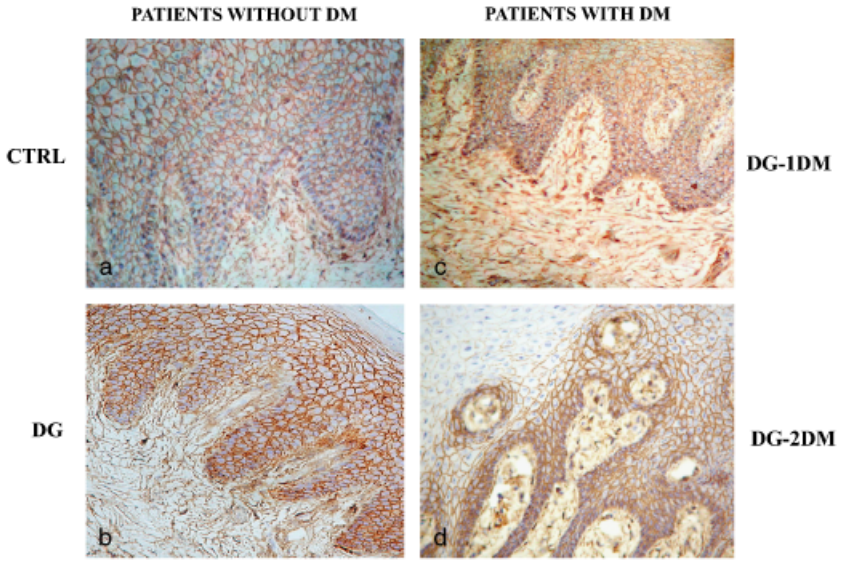

Fig. 2. Immunohistochemical CD44 staining of (a) healthy gingiva (CTRL) (immunoperoxidase; 250 × original magnification), (b) diseased gingival (DG) of patients without diabetes mellitus (DM) (immunoperoxidase; 150 × original magnification), (c) DG of patients with type 1 DM (immunoperoxidase; 150 × original magnification), (d) DG of patients with type 2 DM (immunoperoxidase; 150 × original magnification).

The relationship between diabetes and periodontal disease has been documented in several studies (Jansson et al. 2006, Lalla 2007, Lim et al. 2007). Diabetic subjects are more likely to develop gingivitis and periodontitis (Lalla 2007), and studies correlating the two diseases have demonstrated a greater prevalence of gingival inflammation or periodontitis in children with type 1 DM than in their non-diabetic peers (Salvi et al. 2005, Lalla et al. 2007).

Lim et al. (2007) noted a significant impact of diabetes on periodontal status. In our study after correction for age, BMI and sex, diabetic patients with periodontitis had the greatest CRP, GI, BOP and PD, whereas CAL was significantly increased only in the DG-1DM group.

Compared with non-DM subjects, the two diabetic groups exhibited significant differences in age (which was greater in DG-2DM) and in GI and BOP (which were more elevated in DG-2DM), confirming previous reports of an increase in these periodontal measures in type 1 DM (Karjalainen & Knuuttila 1996).

An important complication of diabetes is vascular changes, with accumulation of high AGE levels in tissues, including periodontium (Martorelli de Lima et al. 2004, Katz et al. 2005). Such changes adversely affect target tissues, especially in terms of collagen stability, amount of HA, endothelial cell function, capillary growth and vessel proliferation (Seibold et al. 1985).

Vascular changes are known to depend on VEGF (Mealey 2006). Guneri et al. (2004) showed VEGF upregulation in diseased periodontal tissue from type 2 DM patients compared with controls. The significantly increased VEGF expression seen in gingival epithelium and endothelial cells of our patients with periodontitis, either with or without DM, lends support to its dysregulation in periodontal disease.

Periodontitis is an inflammatory disease that induces periodontal tissue connective matrix destruction, loss of fibrous attachment, alveolar bone resorption and impaired new bone formation (Silva et al. 2008). Inflammatory reactions are usually characterized by cell accumulation in the extravascular connective tissue. At such sites, inappropriate lymphocyte activation becomes self-perpetuating and can lead to chronic tissue destruction. In addition, locally infiltrated lymphocytes can interact directly with fibroblasts, compromising the connective tissue itself. Understanding the molecular mechanisms involved in cell–cell and cell–matrix interactions is thus of utmost importance (Murakami & Okada 1997).

CD44 seems to be implicated in cellular matrix changes. Several studies have demonstrated that CD44 has a key role in mediating cell–cell and cell–substrate interactions in the periodontium. Leonardi et al. (2006) found that in periodontium, CD44 is involved in

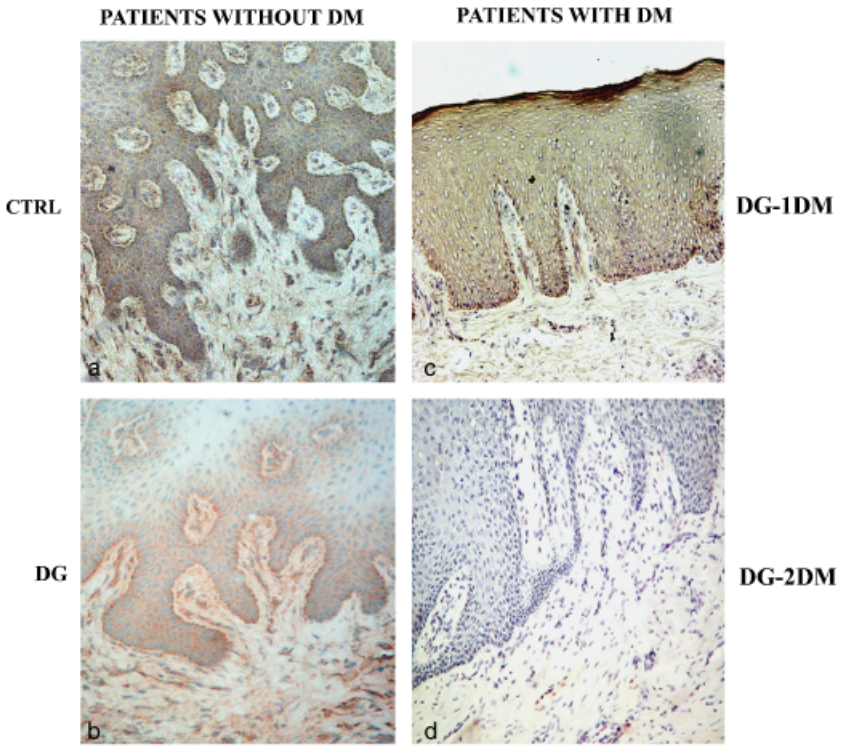

Fig. 3. Immunohistochemical CD133 staining of (a) healthy gingiva (CTRL) (immunoperoxidase; 150 × original magnification), (b) diseased gingival (DG) of patients without diabetes mellitus (DM) (immunoperoxidase; 150 × original magnification), (c) DG of patients with type 1 DM (immunoperoxidase; 100 × original magnification), (d) DG of patients with type 2 DM (immunoperoxidase; 150 × original magnification).

tooth cell differentiation, in cell–matrix interactions and in the accumulation of inflammatory cells in the extravascular connective tissue. CD44 is also the major HA receptor on the keratinocyte surface. During inflammation, HA stimulates immune cells and enhances regulator cytokines of the inflammatory process through an interaction with CD44 and TLR4 receptors. Low-molecular-weight fragments deliver tissue damage signals and mobilize immune cells (Krasiñski & Tchòrzewski 2007). Thus, the interaction between HA and its receptor CD44 initiates inflammatory responses, maintains structural cell integrity and promotes recovery from tissue injury (Jiang et al. 2007).

Murakami et al. (1997) found strong CD44 expression by lymphocytes in gingival connective tissue with periodontal disease.

Overall, these data suggest that the adhesive interaction between lymphocytes and gingival fibroblasts is mediated by the CD44-HA complex (Oksala et al. 1997), which induces intra-cellular signal cascades and regulates cell–cell adhesion and cell migration and proliferation, required for morphogenesis and tissue repair (Bourguignon et al. 2006).

Whereas epithelial CD44 expression was similar in all patients, CD44-positive cells, particularly lymphocytes, were increased in connective tissue from DM patients. These data agree with the literature, confirming the important role of CD44-positive cells in cell adhesion, especially to periodontal extracellular matrix components (e.g. fibronectin, laminin, collagen and HA) (Häkkinen et al. 1993), and recognizing an essential role for the CD44-HA complex in chronic inflammation.

In pathological periodontium, more than in any other tissue, stem cell identification is important not only to evaluate the regeneration process but also to devise new therapies.

CD133 has recently been proposed as a universal marker for tissue stem/progenitor cells (Bussolati et al. 2005). Healing of the periodontal ligament in animal models involves an increased number of progenitor cells in the paravascular area, which induces subsequent migration to the alveolar bone. Thus, inflammatory changes in the periodontium may influence stem cell distribution (Chen et al. 2006).

Our data showed non-significant differences in CD133 expression, especially in periodontal epithelium and vessels, except in type 2 DM patients, where CD133 expression was faint or null. These findings are in line with reports that type 2 DM patients are mostly elderly people (Meneilly & Tessier 2001, Chau & Edelman 2001) with the distinctive tissue structural characteristics of ageing (Ferrari et al. 2007).

The prospects for periodontal tissue regeneration strategies are dependent on our ability to facilitate the repopulation of periodontal lesions by cells capable of promoting regeneration (Ivanovski et al. 2006, Schatteman & Ma 2006). In this respect, therapeutic protocols using CD133-positive stem cells could be associated with biomolecules such as HA (CD44 receptor). Because stem cells lose their therapeutic potential with age (Schatteman & Ma 2006), therapeutic protocols based on them could be especially effective in younger diabetic patients, who have a more favourable periodontal environment and stem cells potential.

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Clinical Relevance

Scientific rationale for the study: The study documents an experimental approach to investigate the expression of the angiogenic and regeneration markers involved in the pathogenesis of periodontal disorders and DM.

Principal findings: The increased values of clinical and periodontal measures underscore the significant impact of diabetes on periodontal status, confirming a role for angiogenic (VEGF) and regeneration (CD44 and CD133) factors in periodontal disease, especially in patients with diabetes.

Practical implications: High VEGF, CD44 and CD133 expression could predict a greater regeneration capacity of gingival tissue and constitute novel targets for efficient stem cell therapy.