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Assessment of vascularity as an index of angiogenesis in periradicular granulomas. Comparison with oral carcinomas and normal tissue counterparts

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Abstract

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Aim To quantify vascularity in periradicular granulomas using different endothelial markers, and assess its value as an index of angiogenesis by comparing granulomas with healthy periodontal ligament (PDL). To use oral tumours, compared with adjacent normal mucosa, as positive controls.

Methodology Paraffin-embedded sections were stained with antibodies to von Willebrand factor (vWF), a pan-endothelial marker, and CD105, a putative marker for angiogenic vessels. Vascularity was quantified by different methods reflecting vessel volume and density.

Results Irrespective of the marker or method used, vascularity values were similar in periradicular granuloma and PDL. Both tissues were highly vascularized, with levels similar to those found in oral squamous cell carcinoma. Vascularity was significantly higher in the latter than in normal mucosa. Fewer vessels were positive for CD105 than for vWF in the normal mucosa, whereas similar numbers were found in the other tissues examined.

Conclusions A comparison of vascularity in oral tumours and normal oral mucosa provided evidence of angiogenesis in the former. Staining with CD105 added limited value to staining with vWF in these tissues. In contrast, a comparison of periradicular granuloma and PDL failed to demonstrate evidence of angiogenesis in the granuloma. As all vessels were similarly stained with vWF and CD105 in granuloma and PDL, a possible hypothesis is that all vessels are newly formed in these tissues. A more plausible alternative is that CD105 expression may reflect the metabolic activity or intrinsic characteristics of the tissues, rather than the presence of angiogenic vessels.

Keywords: angiogenesis, CD105, oral cancer, periodontal ligament, periradicular granuloma, vascularity.

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Introduction

The periodontal ligament (PDL) is a highly cellular specialized connective tissue linking the root cementum to the alveolar bone (Freezer & Sims 1987, Beertsen *et al.* 1997, Mjör & Heyeraas 1998). Periradicular granulomas (PG) are benign growths believed to arise as a reparative response to low-grade periradicular chronic inflammation (Kakehashi *et al.* 1966, Stashenko 1998). PG are anatomically circumscribed lesions which typically contain a heterogeneous collection of vessels, fibroblastoid cells, inflammatory cells and occasionally, epithelial cells derived from the epithelial

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cells rests of Malassez (Nair 2004). At their periphery, PG are continuous with the healthy PDL (Newman & Challacombe 1995).

Periradicular granulomas contain extensive capillary networks, which are presumed to arise by angiogenesis. Angiogenic factors such as fibroblast growth factor-2, tumour necrosis factor-α, transforming growth factor-α, transforming growth factor-β_1 and vascular endothelial growth factor (VEGF), as well as epidermal growth factor receptor have been detected in PG (Lin *et al.* 1996, Tyler *et al.* 1999, Danin *et al.* 2000, Leonardi *et al.* 2003, Moldauer *et al.* 2006). The presence of angiogenic factors in PG and other oral granulomatous lesions (Bragado *et al.* 1999, Yuan *et al.* 2000) is considered to provide evidence of angiogenesis in these tissues.

Angiogenesis is a dynamic process and is consequently difficult to assess with 'one-off' tissue biopsies (as provided by this and other clinical studies) in which conclusions are commonly inferential in nature. To deal with this inherent difficulty, it is important, whenever possible, to compare a lesion with its normal tissue counterpart, from where the lesion originates. As angiogenesis is known to be controlled by the balance between various stimulators and inhibitors, the presence of angiogenic factors in the lesion does not necessarily mean that angiogenesis is taking place (Carlile *et al.* 2001, Harada *et al.* 2001). Therefore, several studies have employed the quantification of blood vessels (vascularity) as an index of angiogenesis (Schor *et al.* 1998a,b). In this case, angiogenesis is inferred by a significant increase in vascularity in the lesion, by comparison to the normal tissue. It has been demonstrated previously that vascularity values increase with neoplastic progression in the oral mucosa, being significantly higher in oral squamous cell carcinoma (OSCC) than in dysplasia, and likewise higher in dysplasia than in normal mucosa (Pazouki *et al.* 1997). These studies were performed using pan-endothelial markers, which stain all blood vessels. A marker specific for newly formed vessels would clearly facilitate the assessment, as it would stain only those tissues containing angiogenic vessels. It has been proposed that CD105 is such a marker (Burrows *et al.* 1995, Schimming & Marme 2002).

The objectives of this study were to (i) compare vascularity in chronic PG and normal PDL, (ii) distinguish between total and new vessels in these tissues and (iii) compare these data with those obtained from OSCC, a tissue known to contain angiogenic vessels, and normal oral mucosa (NOM). Three methods were used to quantify vascularity: Microvascular volume (MVV), average-microvascular density (MVD) and highest-microvascular density (h-MVD) (Chandrachud *et al.* 1997). Immunohistological staining of blood vessels was carried out with antibodies to von Willebrand factor (vWF), a pan-endothelial marker expected to stain all blood vessels and CD105, a putative marker for angiogenic vessels (Burrows *et al.* 1995). To our knowledge, a direct comparison between vascular expression of vWF and CD105 has not been reported previously.

Materials and methods

Specimens

A total of 44 formalin-fixed paraffin-embedded sections were obtained from either the archives of the Oral Pathology Laboratory, University of Dundee or from the University of Münster, Germany. These samples were collected under the Ethics regulations current at the time. The specimens included 13 chronic PG, obtained after extraction or periradicular surgery, and 13 normal PDL controls obtained from the same extracted teeth. In addition, four PDL specimens from adjacent non-endodontically involved teeth were also examined. Histologically, the chronic PG specimens consisted of granulomatous tissue containing varying degrees of inflammatory cell infiltrate (lymphocytes, plasma cells and macrophages), fibroblasts, vessels and in some specimens epithelial cells. A further 14 OSCC specimens obtained from University of Münster, Germany, were used as a positive control (i.e. a tissue known to have high angiogenic activity). Six of the oral carcinoma specimens showed histologically normal peri-tumour tissue adjacent to the tumours (NOM). This normal tissue was used as a control for the carcinomas. Consecutive sections from the middle third of each specimen were stained with vWF and CD105 antibodies alternatively. At least two sections from each specimen were stained with each antibody. A limited number (up to six) of random sections from other areas of the specimens were also examined for a small number (n = 10) of specimens. No significant histological variation was observed between the replicate sections.

Immunohistochemistry

Sections (5 μm) were mounted on silane-coated slides (Superfrost microscope slides; Shandon, Cheshire, UK)

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and stained with antibodies to vWF, a pan-endothelial marker, and endoglin (CD105), a putative marker for angiogenic vessels. A standard immunohistochemistry protocol was followed (Schor *et al.* 1998b). Pre-treatments were required to enhance antigen retrieval: (i) for vWF a 20 min 37 °C incubation using 0.01% protease XXIV diluted in PBS (Sigma, Gillingham, UK) and (ii) for CD105 a 30 min 126 °C autoclave in citrate buffer (0.1 mol L^{-1} pH6). The primary antibodies were: (i) rabbit anti-human vWF (DAKO, Glostrup, Denmark) diluted 1 : 3000 in PBS and (ii) mouse monoclonal anti-human CD105 (Novocasta, Newcastle upon Tyne, UK) diluted 1 : 75 in PBS. Appropriate non-immune IgG (rabbit for vWF, mouse for CD105) (Dako) were used as negative controls. The secondary antibodies (Vector Labs Ltd, Burlingame, CA, USA) were: (i) for vWF a biotinylated goat anti-rabbit antibody and (ii) for CD105 a biotinylated goat anti-mouse antibody.

Quantification of vascularity

Vessels stained with vWF and CD105 were quantified by light microscopy using three methods (Chandrachud *et al.* 1997, Schor *et al.* 1998b):

(1) *Microvascular volume*. It was calculated by a standard stereological method, using a 25 point eyepiece graticule at ×400 magnification. Vessel walls which coincided with the grid points were counted in 15 random fields per section (375 points) and the results expressed as percentage, taking the mean (± standard deviation) for the 15 fields.

(2) *Average-microvascular density*. Using the same grid, magnification and number of random fields, all stained vessels that fell within the area of the grid were counted. Vessels which touched the margins of the grid were only counted if they touched the upper or right-hand margins. The results were converted to mean vessels per mm^2 (± standard deviation) for the 15 fields.

(3) *Highest-microvascular density*. The area of h-MVD was located by scanning the section at ×100 magnification. Three separate fields were counted at ×400 magnification and the mean value was taken as the h-MVD and expressed as the number of vessels per mm. Vascular hot spots were found in OSCC and NOM, but not in PG and PDL sections. Therefore, h-MVD was calculated in the latter as the mean of the highest three MVD field values found per section.

Following initial training, the study sections were quantified by two to four independent observers and any sections with an inter-observer variation above 15% were recounted and agreed by consensus. Intra-observer variation was checked by one observer (KJD) recounting all the sections on two separate occasions.

The intensity of the staining was graded as weak (+), moderate (++), strong (+++) or no staining (−), by comparison to calibration slides.

Assessment of inflammatory infiltration

The extent of inflammatory infiltration of each section was independently assessed by two examiners using four calibration sections. The sections were scored either as grade 0 (no inflammatory infiltrate), grade 1 (mild degree of inflammatory infiltrate), grade 2 (moderate degree of inflammatory infiltrate) or grade 3 (severe inflammatory infiltrate). The final scores were agreed by consensus.

Statistical analysis

Data were analysed using nonparametric tests with SPSS package, version 14 (SPSS Inc., Chicago, IL, USA). Comparisons between any two groups were carried out using Mann–Whitney *U*-test and correlations using Spearman rank correlation. The Chi-square test was used to compare frequencies. Intra- and inter-observer variation was analysed using Wilcoxon paired sample test. Results were considered significant at the 95% level of confidence ($P < 0.05$).

Results

Visualization of the blood vessels

Antibodies to vWF and CD105 were used to stain four types of tissue: (i) PG, (ii) normal PDL, (iii) OSCC and (iv) histologically NOM adjacent to tumour. Good positive staining of blood vessels, with minimal background, was achieved with both antibodies. Representative examples are shown in Fig. 1. The intensity of the staining was similar with both antibodies in PG and PDL, whereas it was generally weaker in NOM than in OSCC (see quantification below). Maximal contrast observed between vWF and CD105 staining in NOM is shown in Fig. 1g,h. Antibody to vWF stained only blood vessels, whereas CD105 antibody stained blood vessels and occasionally cells of the inflammatory infiltrate, as in Fig. 1f. These were easily distinguished from blood vessels and therefore did not affect the quantification of the latter. Control sections, stained

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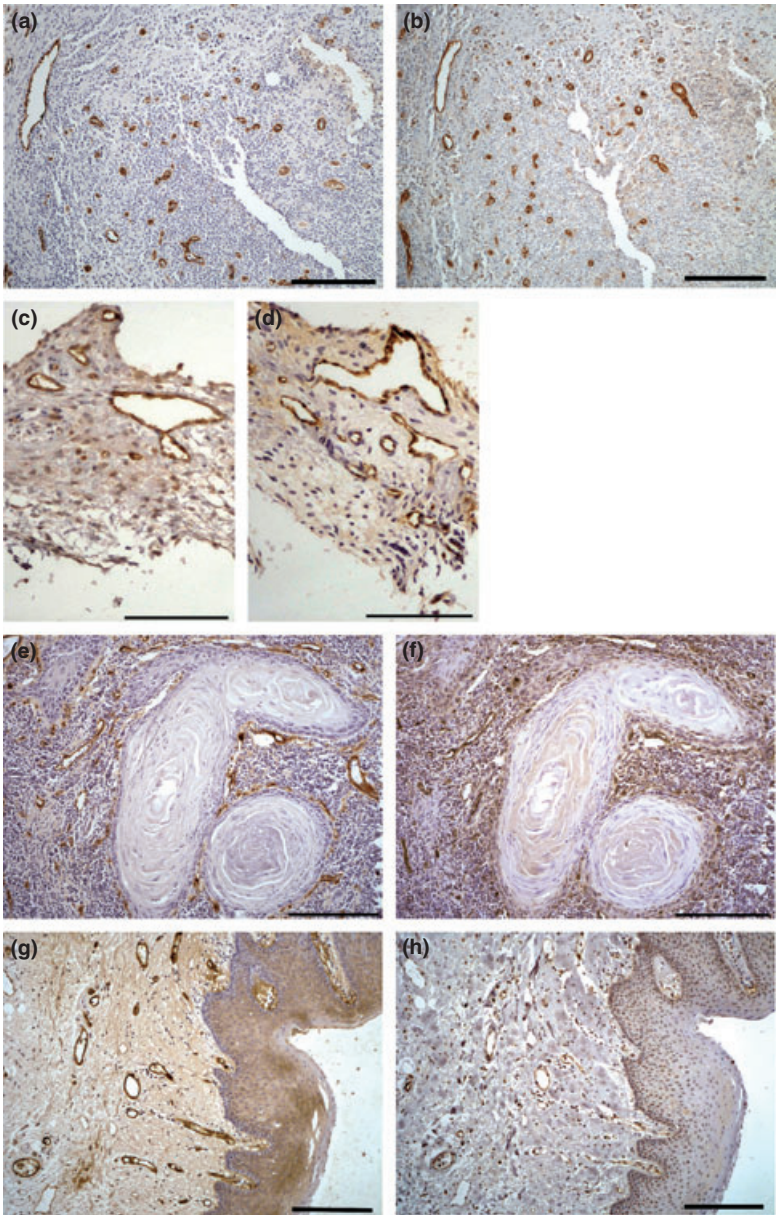

Figure 1 Sections of oral tissues stained with von Willebrand factor (a,c,e and g) and CD105 (b,d,f and h). (a,b) Periradicular granuloma; (c,d) Periodontal ligament; (e,f) Oral squamous cell carcinoma; (g,h) Peri-tumour normal oral mucosa. Scale bar: 50 μm (a–f) and 100 μm (g) and (h).

with the corresponding nonimmune IgG, were always negative.

Assessment of the methodology

Vessels stained with vWF and CD105 antibodies were quantified by three different methods: MVV, MVD and h-MVD. Results were obtained by consensus of two to three trained independent observers. Intra- and inter-observer variations were not significant, with *P*-values ranging from 0.423 to 0.875. Volume and density vascularity scores were directly and significantly correlated for all tissues and antibodies, with correlation coefficients ranging from 0.523 to 0.911 and *P*-values at the 99% level.

Comparison of OSCC and peri-tumour NOM tissues

Vascularity in OSCC has been reported to be significantly higher than in the corresponding NOM (Pazouki *et al.* 1997, Schimming & Marme 2002, Li *et al.* 2005). Therefore, these tissues represent a positive model of

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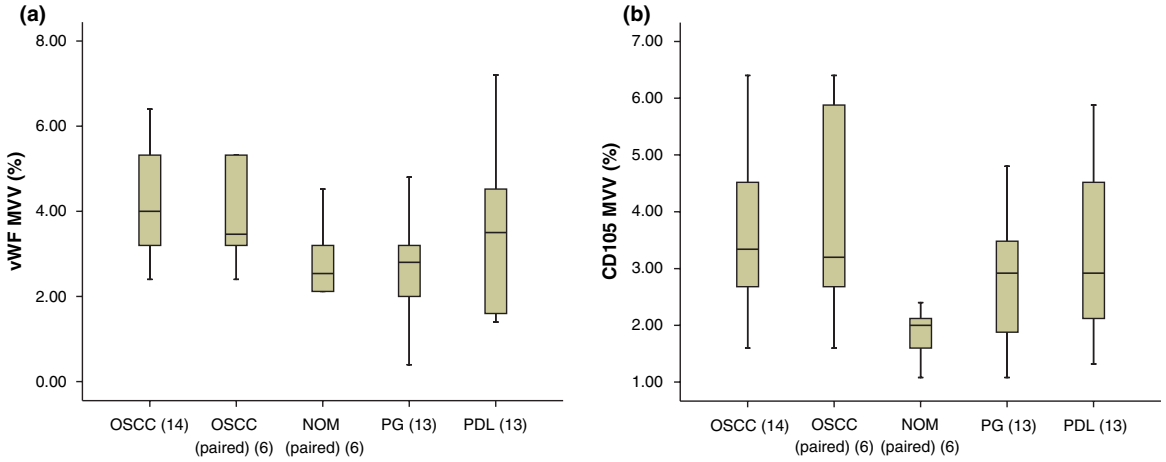

Figure 2 Estimate of microvascular volume (%) for the different tissues using (a) vWF and (b) CD105. The box represents the interquartile range, the horizontal line the median and the whiskers the full range. OSCC, oral squamous cell carcinoma; NOM, normal oral mucosa, PG, periradicular granuloma, PDL, periodontal ligament; vWF, von Willebrand factor.

angiogenesis. In this study, consecutive sections were stained with antibodies to vWF, a pan-endothelial marker and to CD105, a putative marker of angiogenic vessels. Vascularity values were significantly higher in OSCC ($n = 14$) than in the NOM tissues ($n = 6$), irrespective of the antibody or quantification method used (P-values ranging from 0.031 to 0.001). Results for MVV (vWF and CD105) are shown in Fig. 2, MVD in Fig. 3 and those for h-MVD in Fig. 4. When comparing only the six OSCC which contained NOM, the difference between paired OSCC and NOM groups was still significant for vWF-MVD ($P = 0.01$), vWF-h-MVD ($P = 0.026$), CD105-MVV ($P = 0.03$) and CD105-h-MVD ($P = 0.002$). The difference was nearly significant (significant at the 90% level) for vWF-MVV ($P = 0.08$) and CD105-MVD ($P = 0.10$). In all cases, vascularity values for the six OSCC were not significantly different than those for the 14 OSCC (P ranges from 0.51 to 0.93). These findings demonstrate the robustness of the methodology, as significant or near significant differences between tumour and normal tissues were detectable even in very small experimental groups.

As mentioned above, the intensity of the staining with CD105 antibody was generally weaker in NOM than in OSCC. Analysis of the six matched specimens

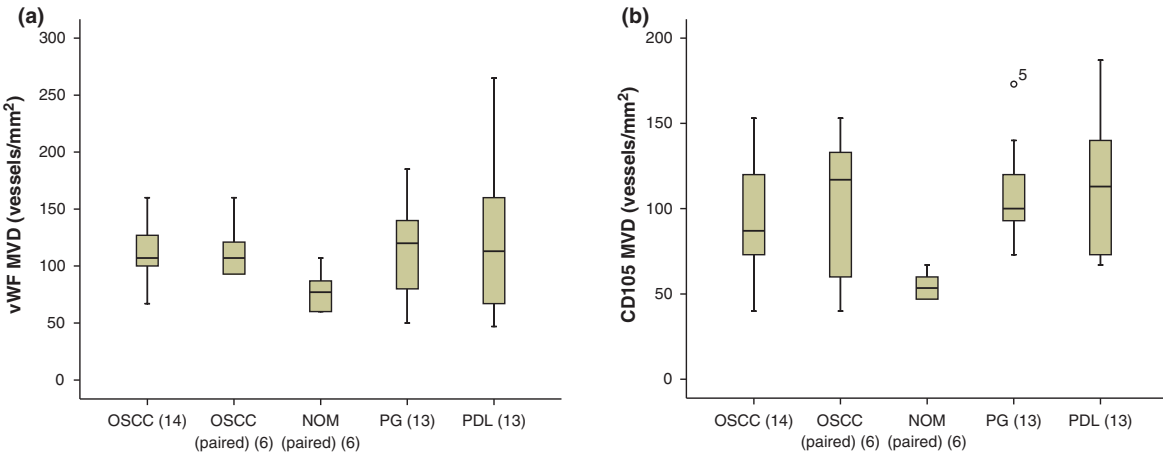

Figure 3 Estimate of microvascular density (MVD, vessels/mm^2) for the different tissues using (a) vWF and (b) CD105. The box represents the interquartile range, the horizontal line the median and the whiskers the full range. OSCC, oral squamous cell carcinoma; NOM, normal oral mucosa; PG, periradicular granuloma; PDL, periodontal ligament; vWF, von Willebrand factor.

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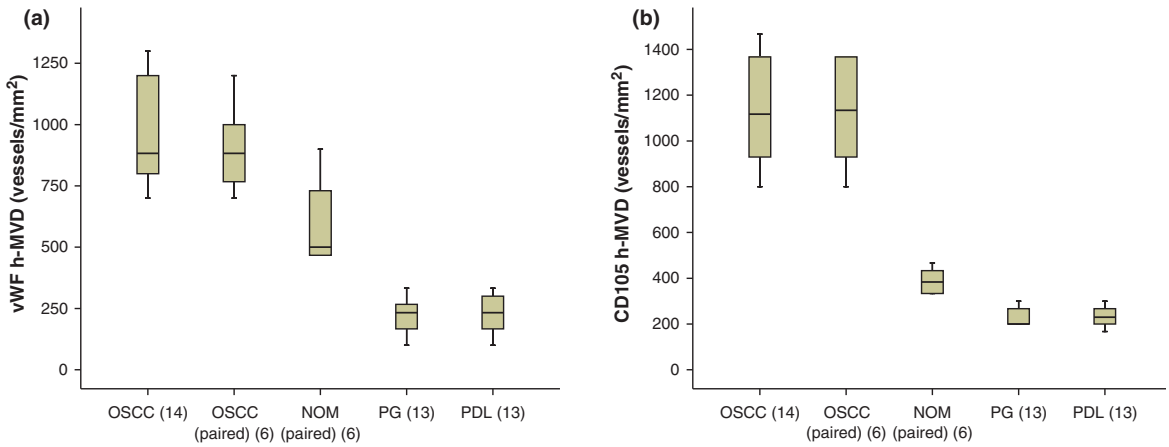

Figure 4 Estimate of highest-microvascular density (h-MVD, vessels/mm^2) for the different tissues using (a) vWF and (b) CD105. The box represents the interquartile range, the horizontal line the median and the whiskers the full range. OSCC, oral squamous cell carcinoma; NOM, normal oral mucosa; PG, periradicular granuloma; PDL, periodontal ligament, vWF, von Willebrand factor.

Table 1 Staining intensity of vessels positive for vWF and CD105

Vascular marker	Tissue	Staining intensity +	++	+++	Chi-square *P*-value
vWF	NOM	2	3	1	0.29
	OSCC	0	4	2	
	PDL	0	0	13	1.0
	PG	0	0	13	
CD105	NOM	5	1	0	0.012*
	OSCC	0	4	2	
	PDL	0	0	13	1.0
	PG	0	0	13	

Comparison between NOM and OSCC and between PDL and PG.
vWF, von Willebrand factor; OSCC, oral squamous cell carcinoma; NOM, normal oral mucosa; PG, periradicular granuloma; PDL, periodontal ligament.
*Significant difference.

Table 2 CD105 vascularity values (MVV, MVD and h-MVD) as a percentage of vWF (mean ± SD)

Tissue group	*n*	MVV	MVD	h-MVD
PG	13	127 ± 97	108 ± 64	112 ± 38
PDL	13	101 ± 31	108 ± 41	116 ± 43
OSCC	14	101 ± 61	86 ± 37	*124 ± 29
Paired OSCC	6	118 ± 89	97 ± 51	**125 ± 26
NOM	6	71 ± 28	71 ± 6	68 ± 13

vWF, von Willebrand factor; MVV, microvascular volume; MVD, microvascular density; h-MVD, highest-microvascular density; OSCC, oral squamous cell carcinoma; NOM, normal oral mucosa; PG, periradicular granuloma; PDL, periodontal ligament.
*h-MVD OSCC > NOM (P = 0.001).
**h-MVD Paired OSCC > NOM (P = 0.002).

revealed that the intensity of vWF-positive vessels was not significantly different in NOM and OSCC, whereas that of CD105-positive vessels was significantly weaker in NOM (Chi-square P = 0.012) (Table 1). When CD105 vascularity values were expressed as a percentage of vWF (Table 2), the percentage was lower in NOM than in OSCC and highest in the 'hot spot' of tumours. The difference between NOM and OSCC was statistically significant only for h-MVD ($P \leq 0.002$).

Comparison of PG and PDL

Vascularity was then assessed in PG (n = 13) and normal PDL (n = 13) tissues using the same six parameters: vWF-MVV, vWF-MVD, vWF-h-MVD, CD105-MVV, CD105-MVD and CD105-h-MVD. Values tended to be higher in the PDL tissues compared with the PG, but the differences were not significant. Results for MVV, MVD and h-MVD are shown in Figs 2, 3 and 4, respectively. There was no difference between PG and PDL specimens in the intensity of vWF and CD 105 staining (Table 1). Furthermore, CD105 values expressed as a percentage of vWF (Table 2) were not different, (approximately 100%) in PG and PDL samples. There was no evidence of a 'field change effect' in the vascularity of the PDL tissue as a result of the adjacent endodontic infection, as vascularity levels in PDL specimens from adjacent non-endodontically involved teeth (n = 4) were not significantly different from those of PDL adjacent to PG (n = 13).

Statistically higher levels of inflammatory infiltrate were found in the PG compared with the PDL tissue

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(Chi-square test, $P = 0.001$; results not shown). No correlation was found between the inflammatory index of the PG and the vascularity scores.

Discussion

Angiogenesis cannot be measured directly in human lesions, but can be inferred by measuring angiogenesis indices (e.g. angiogenic factor expression, vascularity) in the lesion of interest and its normal tissue counterpart (see Introduction). In this study, vascularity was assessed in four different types of tissue: periradicular granuloma (PG), normal periodontal ligament (PDL), oral squamous cell carcinoma (OSCC), and peri-tumour histologically normal oral mucosa (NOM). Vessels were stained with antibodies to von Willebrand factor (vWF) and CD105 and quantified by three methods that reflect vascular volume and density. These were average-microvascular volume (MVV), average-microvascular density (MVD) and highest-microvascular density (h-MVD). Each lesion was compared with its corresponding normal tissue, thus representing two different models (PG-PDL and OSCC-NOM) that can be compared with each other. Two main questions are addressed by this study: (i) is there evidence of angiogenesis in chronic PG? and (ii) is CD105 a marker of angiogenic vessels?

Vascularity was significantly higher in OSCC than in NOM indicating that angiogenesis accompanies the development of the former. This finding confirms the validity of the OSCC-NOM model as a positive control (Pazouki *et al.* 1997) and is consistent with the concept of tumour growth being angiogenesis-dependent in order to meet the increased metabolic requirements of the tumour (Folkman 1971). In contrast, there was no difference in vascularity between PG and PDL tissues, irrespective of the antibody or methodology used. Therefore, there is no evidence of angiogenesis taking place in the PG-PDL model. This finding was unexpected as it had been previously suggested that the formation of granulomatous lesions is accompanied by angiogenesis (see Introduction). Therefore, it was anticipated that a presumably angiogenic PG would have higher vascularity levels than the PDL. Information obtained from tissue sections is applicable to a fixed point in time. It cannot be excluded, therefore, the possibility that angiogenesis may have occurred at earlier stages of PG development. This could be examined in animal models, but it is not possible to determine in humans, as only chronic lesions are clinically detectable and accessible for ex-vivo experimentation. Nevertheless, chronic oral lesions, such as oral lichen planus (Tao *et al.* 2007), have been found to contain significantly higher vascularity than the corresponding normal tissue. Sampling error is another potential problem, giving the heterogeneity of the tissues examined. However, no significant difference in vascularity values were found when up to six replicate sections of PG and PDL specimens were assessed. Furthermore, no significant differences in vascularity were found in the OSCC-NOM model, in spite of the well-known heterogeneity of the tumours.

Various methods have been used to quantify vascularity, the most common being the h-MVD in which only the most vascularized area of the section (hot spot) is assessed (Weidner *et al.* 1991). Alternative methods involve estimating MVD or volume (MVV) in randomly selected areas of the sections (Chandrachud *et al.* 1997, Pazouki *et al.* 1997). Previous findings have demonstrated that different results may be obtained depending on the quantification method used (Pazouki *et al.* 1997, Schor *et al.* 1998b, Li *et al.* 2005). For example, MVV increased significantly, in a step-wise fashion, with disease progression in oral lesions, from NOM to dysplastic lesions and carcinomas, whereas h-MVD did not discriminate between dysplasias and carcinomas (Pazouki *et al.* 1997). Similarly, Li *et al.* (2005) found that h-MVD in CD31 stained sections could not differentiate between NOM and dysplastic lesions, but did significantly differentiate between NOM and oral carcinoma. It is important, therefore to assess vascularity by more than one method.

The vessels were homogeneously distributed in PG and PDL, and there was no evidence of vascular hot spots. Nevertheless, the highest-MVD, as well as the average-MVD and MVV were assessed in conjunction with vWF and CD105 staining to allow direct comparison between the OSCC-NOM and the PG-PDL models. Confidence in the methodology adopted was demonstrated by: (i) the lack of significant inter- and intra-observer variations, (ii) the significant correlation between h-MVD, MVV and MVD values and (iii) the significant difference between OSCC and NOM tissues. These latter results are in agreement with previous publications using larger numbers of specimens (Pazouki *et al.* 1997, Schimming & Marme 2002, Li *et al.* 2005).

It is widely assumed that angiogenesis is involved in the formation of PG. Evidence supporting this assumption includes the presence of various angiogenic factors in these lesions (see Introduction). As angiogenesis cannot be measured directly in human tissues, expression of such factors is taken as an index of angiogenesis. However, angiogenesis is regulated by a complex

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network of stimulators and inhibitors, including both soluble factors and insoluble extra-cellular matrix components. The expression of an angiogenic factor, therefore, does not necessarily indicate that angiogenesis has taken place, as the environment may not be permissive for angiogenic activity. In PG, the expression of angiogenic factors has been studied in relation to the level of immune cell infiltration and/or the extent of epithelial proliferation, but not to the vascularity of the lesions (Lin *et al.* 1996, Tyler *et al.* 1999, Danin *et al.* 2000, Leonardi *et al.* 2003, Moldauer *et al.* 2006). Quantification of vascularity represents another index of angiogenesis. In all cases, it is important to test the robustness of an angiogenic index by comparing an assumed angiogenic tissue with its closest non-angiogenic counterpart. For example, expression of VEGF, a potent angiogenic factor, was found to be similar or higher in NOM than in OSCC, therefore it cannot be taken as a reliable index of angiogenesis in OSCC (Baillie *et al.* 2001). In contrast, vascularity appears to represent a good index of angiogenesis in oral tissues (Pazouki *et al.* 1997, Schimming & Marme 2002, Li *et al.* 2005). It should be noted that, when using pan-endothelial markers, not all tumours have higher vascularity than the normal tissue from which they originate (Schor *et al.* 1998a,b). In such cases, it is not possible to determine whether angiogenesis has taken place.

Pan-endothelial markers such as CD34, CD31 and vWF do not distinguish between mature and newly formed (or angiogenic) vessels. Furthermore, it has been reported that such markers react strongly with endothelial cells in large blood vessels but their expression is weak or absent in microvessels in most normal tissue and in many tumours (Wang *et al.* 1994). Although no evidence was found in the present study of vessels not expressing vWF, the use of a marker specific for angiogenic vessels would be preferable to provide an accurate measure of angiogenesis. CD105 (endoglin) has been reported to be such a marker, being selectively expressed by proliferating endothelial cells *in vitro* and angiogenic vessels in tumours (Burrows *et al.* 1995), including OSCC (Schimming & Marme 2002). Furthermore, high expression of CD105 in tumours has been associated with poor prognosis, being more informative than pan-endothelial markers (Kumar *et al.* 1999, Tanaka *et al.* 2001, Chien *et al.* 2006, Kyzas *et al.* 2006).

Unexpectedly, CD105 stained vessel endothelial cells in a similar fashion to vWF in PG and PDL, and did not provide any additional information in these tissues. In the OSCC-NOM model, staining with CD105 added very limited value to staining with vWF. These results agree with those of Balza *et al.*(2001) who found CD105-positive vessels in a variety of normal (non oral) human tissues. CD105 has been previously shown to stain vessels in periradicular granulation tissue, although no attempt was made to quantify or compare this with other tissues (Tasman *et al.* 2000). In NOM, CD105 expression was weaker than in OSCC regarding intensity of staining. When expressed as a percentage of vWF-positive vessels, CD105-positive vessels were also significantly lower in NOM than in OSCC when measured by h-MVD, but not by MVV or MVD. Otherwise, there were no significant differences in the four tissues examined, although they tended to be similarly high in PG, PDL and OSCC and lower in NOM. The hypothesis that CD105 is a specific marker for angiogenic vessels has not been proved. If this hypothesis were correct, the present results would indicate that angiogenesis is taking place in all the four tissues examined, at similar high levels in OSCC, PG and PDL and at lower levels in NOM. However, an alternative hypothesis can be proposed, namely that CD105 may be associated with high tissue turnover or some other intrinsic characteristic of these tissues, rather than with the formation of new blood vessels.

Conclusions

Periradicular granulomas have previously been shown to express angiogenic factors. In this study, vascularity, rather than angiogenic factor expression, has been used as an alternative and more direct index of angiogenesis ex-vivo. OSCC were used as positive controls and tissues believed to contain newly formed (angiogenic) vessels (i.e. PG, OSCC) were compared with their normal tissue counterparts. CD105 has been reported to be expressed by angiogenic, but not by mature vessels, whereas all vessels express vWF. Various methods used to quantify vascularity in histological sections may lead to different results.

Irrespective of the method or marker (vWF, CD105) used, similar vascularity levels were found in PG and histologically normal PDL. In contrast, significantly higher levels of vascularity were found in OSCC than in adjacent NOM. Therefore, a comparison of OSCC and NOM indicate that angiogenesis has occurred in the development of OSCC. In contrast, a comparison of PG and PDL failed to show evidence of angiogenesis taking place in these tissues.

The results do not provide evidence that CD105 is a marker of angiogenic vessels, but may be explained

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according to two contrasting hypothesis: (i) angiogenesis is taking place in all the four tissues examined, at similar high-levels in OSCC, PG and PDL and at a slightly lower level in NOM, and (ii) CD105 may be associated with some intrinsic characteristic of these tissues (e.g. high metabolic activity), rather than with the presence of angiogenic vessels.

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