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Mechanisms of action of environmental factors – tobacco smoking

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Abstract

Aim: To review the potential biological mechanisms underlying the effects of tobacco smoking on periodontitis.

Main findings: Smoking has major effects on the host response, but there are also a number of studies that show some microbiological differences between smokers and non-smokers.

Smoking has a long-term chronic effect on many important aspects of the inflammatory and immune responses. Histological studies have shown alterations in the vasculature of the periodontal tissues in smokers. Smoking induces a significant systemic neutrophilia, but neutrophil transmigration across the periodontal microvasculature is impeded. The suppression of neutrophil cell spreading, chemokinesis, chemotaxis and phagocytosis have been described. Protease release from neutrophils may be an important mechanism in tissue destruction. Tobacco smoke has been found to affect both cell-mediated immunity and humoral immunity. Research on gingival crevicular fluid has demonstrated that there are lower levels of cytokines, enzymes and possibly polymorphonuclear cells in smokers. In vitro studies have shown detrimental effects of nicotine and some other tobacco compounds on fibroblast function, including fibroblast proliferation, adhesion to root surfaces and cytotoxicity.

Conclusion: Tobacco smoking has widespread systemic effects, many of which may provide mechanisms for the increased susceptibility to periodontitis and the poorer response to treatment.

Key words: smoking; periodontitis; bacteria; neutrophils; lymphocytes; fibroblasts

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Tobacco smoking, mostly in the form of cigarette smoking, is recognized as the most important environmental risk factor in periodontitis, and, with respect to this, there are large number of supporting epidemiological studies which will not be the focus of this review. Over the last decade, there have also been a number of excellent reviews that have considered the biological basis and pathogenic mechanisms in addition to the clinical and epidemiological aspects (Barbour et al. 1997, Tonetti 1998, Kinane & Chestnutt 2000, Johnson & Hill 2004, Mullally 2004). It has usually been necessary to rely on the vast body of medical literature that describes the noxious effects of smoking, and to apply this knowledge to the pathogenesis of periodontitis. Therefore, many of these mechanisms may be implied or speculative. This review will attempt to build on previous reviews in the periodontal literature and make attempts to primarily consider evidence that directly pertains to the periodontal tissues, citing more general papers only where necessary.

It is fair to say that much of the work in the periodontal and medical literature has concentrated on the effects of nicotine in tobacco. While nicotine is the primary psychoactive component, and addiction to it is the main reason for people subjecting themselves to frequent and high doses over many years, one must appreciate that tobacco smoke contains thousands of different compounds (Table 1). Many of these are directly noxious/poisonous to living organisms and cells, and nicotine may be unfairly blamed for most of these properties. Moreover, it is also very important to appreciate that most of the harmful effects of tobacco products will result from systemic exposure through absorption in the lungs rather than topical absorption in the oral cavity (Palmer et al. 2000).

A regular heavy smoker exposes himself/herself to these compounds many times per day for several minutes at a time. Although increasing evidence is being presented for the harmful effects of passive smoking, the periodontal

Table 1. Some constituents of tobacco smoke (http://www.ash.org.uk/)

Particulate phase	Gas phase
Nicotine	Carbon monoxide
''Tar'' (composed of many chemicals)	Ammonia
Benzene	Dimethylnitrosamine
Benzo(a)pyrene	Formaldehyde
	Hydrogen cyanide
	Acrolein

literature is generally confined to active smoking. Many smokers develop the habit in their teenage years and continue it throughout their life. No other drug is administered so frequently or over such a time period as smoking. This is to emphasize the fact that the detrimental effects on the periodontium are derived from long-term chronic exposure and bear little relationship with the effects that can be measured on a single exposure. The importance of measurement and validation of exposure to tobacco smoke is considered in the review by Scott et al. (2001). Cotinine, a metabolite of nicotine, can be measured in the serum/plasma and saliva, and is a better measure of tobacco smoke exposure as it has a longer half-life than nicotine (18 h compared with 1–2 h). Smokers would be expected to have serum cotinine levels of over 14 ng/ml, and this could be as high as 1000 ng/ml. Resting plasma nicotine levels are much lower (5–50 ng/ml), and are maintained by the individual to satisfy their craving for nicotine. Because nicotine is so rapidly absorbed from the lung and transport to the brain is rapid, very high peak levels can be measured in the brain. It is important to understand these variations in relation to levels tested in in vitro experiments.

Tobacco smoking affects the oral environment and ecology, the gingival tissues and vasculature, the inflammatory response, the immune response and the homeostasis and healing potential of the periodontal connective tissues. This review will follow this line and will clearly differentiate between the effects of whole tobacco smoke and nicotine and chronic and acute exposure where appropriate.

Effect of Smoking on Plaque

Effect of smoking on plaque development

Early observational reports that smokers showed a higher prevalence of dental plaque than non-smokers suggested that more severe periodontal disease in smokers might be because of greater accumulations of plaque (Kristoffersen 1970, Preber et al. 1980). However, other studies indicated that, when controlling for other factors, smoking did not appear to increase the amount of plaque (Alexander 1970, Sheiham 1971). In addition, studies in which the development of plaque and inflammation was observed in an experimental gingivitis model showed that the rate of plaque formation was similar between smokers and non-smokers (Bastiaan & Waite 1978, Bergstrom 1981, Bergstrom & Preber 1986, Lie et al. 1998a).

Effect of smoking on the oral flora

Studies of the relationship between smoking and the oral flora are limited in comparison with those that have established a link with more severe periodontal disease. The majority of studies have investigated the difference in the subgingival microflora between smoking and non-smoking subjects with periodontal disease (Table 2). However, a study of the microbiota of the oral mucous membranes and saliva failed to establish a statistically significant trend for smokers to harbour greater proportions of putative periodontal pathogens in these oral locations (Mager et al. 2003). Similarly, an experimental gingivitis study showed no differences between smokers and non-smokers in the alterations to supra- and subgingival microflora during the change from relative health to experimentally induced gingivitis (Lie et al. 1998b). However, a study of the microflora of the gingival crevice in 25 smokers and 25 non-smokers with a relatively healthy periodontium showed a higher prevalence of infection with at least one of the tested pathogens in smokers (Shiloah et al. 2000).

Effect of smoking on the subgingival microflora in periodontitis

The vast majority of studies of the influence of smoking on the periodontal microflora have been cross-sectional investigations of patients with chronic periodontitis (Table 2). Given the convincing evidence for differences in the clinical and immunological status of the subgingival environment in smokers and non-smokers, it would be reasonable to propose that they should exhibit differences in the microflora. However, a number of studies have indicated that smoking has little effect on the subgingival microflora. Preber et al. (1992) sampled a single site with a probing pocket depth $\geqslant$6 mm and compared 83 smokers and 62 non-smokers for the presence and proportion of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* using cultural methods. They showed no significant differences between the two groups of subjects. A more recent cultural study (van der Velden et al. 2003) showed similar results. The authors reported sampling the deepest sites in four quadrants and analysing for *A. actinomycetemcomitans*, *Tannerella forsythensis* (*Bacteroides forsythus*), *Fusobacterium nucleatum*, *P. gingivalis*, *P. intermedia* and *Micromonas micros* (*Peptostreptococcus micros*) before and after treatment. No significant differences in the prevalence of the various bacteria were seen between 30 smokers and 29 non-smokers before treatment. Stoltenberg et al. (1993) used an immunofluorescent method to examine eight samples per subject to establish the presence or absence of *A. actinomycetemcomitans*, *Eikenella corrodens*, *F. nucleatum*, *P. gingivalis* and *P. intermedia*. No significant differences were reported between the 63 smokers and 126 non-smokers, although the presence of some test bacteria and smoking were separately associated with increased risk of probing depths $\geqslant$3.5 mm. Two recent studies have used DNA identification techniques to investigate differences between smokers and non-smokers. Darby et al. (2000) investigated the prevalence of *A. actinomycetemcomitans*, *T. forsythensis*, *P. gingivalis*, *P. intermedia* and *Treponema denticola* in 10 smoking and 23 non-smoking patients with adult periodontitis using PCR. The authors also reported findings from 12 smokers and 12 non-smokers with generalized early-onset periodontitis. In neither group did they observe differences in the microflora which could be attributed to smoking. Bostrom et al. (2001) used checkerboard DNA–DNA hybridization technology to detect the presence of a wide range of species in 33 smokers and 31 non-smokers. No influence of smoking on the occurrence of any species was observed.

The earliest reported evidence of microbiological differences between

Table 2. Microbiological studies of periodontitis in smokers and non-smokers

References	Number of smokers/non-smokers*	Smoking history	Sampling and sites	Laboratory procedures	Organisms investigated included	Results: smokers *versus* non-smokers
Mager et al. (2003)	47/182 84 subjects periodontally healthy	ND	Swab eight oral mucous membrane sites	DNA	*Pg, Pi, Mm, Si, Sn, Td* and others	Non-significant trends to higher levels of pathogens in smokers
van der Velden et al. (2003)	30/29	Cotinine $\geqslant$ 5ng/ml	Paper points to four deepest sites	Culture	*Aa, Tf, Fn, Pg, Pi, Mm*	No difference
Bostrom et al. (2001)	33/31	$\geqslant$ 20 cig/day $\geqslant$ 32 years	Paper points to four sites $\geqslant$ 5 mm	DNA	*Aa, Tf, Cr, Ec, Fn, Pg, Pi, Mm, Pn, Si, Sn, Td*	No difference
Eggert et al. (2001)	86/163	ND‡	Paper points to two deepest sites	IA	*Aa, Pg, Pi*	Higher proportions of *Pi* in smokers
Haffajee and Socransky (2001)	50/124	ND	Curette Mesio-buccal of all teeth	DNA	*Aa, Cr, Csp, Ec, En, Fn, Pi, Mm, Pn, Tf, Pg, Si, Sn, Td*	Higher prevalence of *Tf, En, Fn, Pi, Mm, Pn, Pg, Td* in smokers at sites $\leqslant$ 4 mm
Van Winkelhoff et al. (2001)	88/90	ND	Paper points to four deepest sites	Culture	*Aa, Tf, Cr, Fn, Pg, Pi, Pn, Mm*	Higher prevalence of *Pi/Pn* and higher proportions of *Fn, Mm* in smokers
Darby et al. (2000)	22/33	ND	Curette four sites $\geqslant$ 5 mm	PCR	*Aa, Tf, Pg, Pi, Td*	No difference
Shiloah et al. (2000)	25/25 *healthy subjects*	$\geqslant$ 15 cig/day $\geqslant$ 3 years	Paper points to healthy sites	DNA	*Aa, Cr, Ec, Fn, Pg, Pi, Td*	Higher prevalence of infection with at least one pathogen in smokers
Kamma et al. (1999)	30/30 early onset periodontitis	$\geqslant$ 20 cig/day	Paper points to two sites $\geqslant$ 5 mm	Culture	*Aa, Tf, Cr, Ec, Fn, Pg, Pi, Mm, Si*	Higher prevalence and proportions of *Tf, Cr, Pg, Mm* and others in smokers
Lie et al. (1998b)	11/14 experimental gingivitis	$\geqslant$7 cig/day	Paper points to pooled supra- and sub-gingival	Culture	*Aa, Cr, Fn, Pg, Pi, Mm and others*	No differences
Umeda et al. (1998)	21/146	ND	Paper points to four deepest sites	PCR	*Aa, Tf, Pg, Pi, Pn, Td*	Higher prevalence of *Td* in smokers
Zambon et al. (1996)	798/628 smokers included former smokers	ND	Paper points two pooled samples from six sites	IF	*Aa, Tf, Cr, Es, Fn, Pg, Pi*	Higher prevalence of *Aa, Tf, Pg* in smokers
Stoltenberg et al. (1993)	63/126	ND	Curette	IF	*Aa, Ec, Fn, Pg, Pi*	No difference
Preber et al. (1992)	83/62	ND	Paper points to one site $\geqslant$ 6 mm	Culture	*Aa, Pg, Pi*	No difference

*All subjects with chronic adult periodontitis unless otherwise stated.

Aa, Actinobacillus actinomycetemcomitans; Cr, Campylobacter rectus; Ec, Eikenella corrodens; En, Eubacteria nodatum; Fn, Fusobacterium nucleatum; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; Mm, Micromonas micros; Pn, Prevotella nigrescens; Sn, Selenomonas noxia; Si, Streptococcus intermedius; Td, Treponema denticola; Tf, Tannerella forsythensis; Csp, Capnocytophaga sputigena; ND, No details; IA, Commercial immunoassay; PCR, Polymerase Chain Reaction; IF, Immunofluorescence; DNA, DNA–DNA Hybridization; cig/day, cigarettes per day

smokers and non-smokers was provided by Zambon et al. (1996). The authors investigated a substantial population of 1426 subjects, of whom 798 were current or former smokers and 628 were never-smokers. Immunofluorescent microscopy was used to identify the presence of a number of putative periodontal pathogens from two pooled samples per subject. The results indicated a higher prevalence of *A. actinomycetemcomitans*, *T. forsythensis* and *P. gingivalis* in the current or former smokers. In particular, the authors reported that the risk of subgingival infection with *T. forsythensis* in current smokers was 2.3 times that of non-smokers. Umeda et al. (1998) carried out a study of risk indicators for harbouring six periodontal pathogens in 199 subjects. Despite including only 21 smokers, their published report stated that current smokers displayed an increased risk (Odds ratio 4.6) for harbouring *T. denticola* in periodontal pockets. No other differences were reported.

Three studies reported findings from reasonably sized sample populations of patients with chronic periodontitis in 2001. Haffajee and Socransky (2001) investigated the prevalence of a large number of species using checkerboard DNA–DNA hybridization in 272 subjects, of whom 50 were current smokers, 98 were past smokers and 124 were never-smokers. They reported a higher prevalence of eight species in current smokers than in the other two groups. Higher prevalence of colonization of periodontal pathogens at shallow sites ($<$4 mm) in current smokers was an important additional observation. Eggert et al. used a commercial kit, which identified the presence of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*, to investigate differences between 86 smokers and 163 non-smokers. Samples were taken from the deepest sites in each patient, either as two single or two pooled samples. Results of the analysis indicated a statistically greater quantity of *P. intermedia* in smokers, but no differences with regard to the other organisms. In the same year, van Winkelhoff and co-workers compared treated and untreated smokers with non-smokers using cultural methods. Samples were taken from the deepest site in each quadrant. The comparison of untreated subjects indicated a higher prevalence of *P. intermedia/P. nigrescens* and a higher

proportion of *F. nucleatum* and *M. micros* in the smokers.

In contrast to the findings of Darby et al. (2000), Kamma et al. (1999) have reported some microbiological differences between 30 smokers and 30 non-smokers with early-onset periodontitis. Plaque collected from the deepest pockets in each quadrant were pooled into two samples and cultured to identify the presence and proportions of nine bacterial species. Their results showed a higher prevalence and proportion of *T. forsythensis, Campylobacter rectus, P. gingivalis* and *M. micros* in samples from smokers.

Conclusions

There are problems associated with microbiological investigations of the oral flora that may affect the interpretation of the results of the studies reviewed here. Estimates of the number of species that regularly colonize the subgingival environment vary between 400 and 600. At least half of these are unculturable and many have yet to be reliably speciated. Inevitably, those whose biotypes have been most extensively characterized are most often investigated. Sampling methods vary widely, and, together with undoubted differences from site to site within the mouth, such variations may affect the results of studies. Identification of organisms by different methods such as culture, immunofluorescence and DNA-based techniques gives rise to potentially different outcomes. Under these circumstances, it is imperative that studies with adequate numbers of subjects are performed in order to overcome the background of extreme variation, which will potentially mask the effects of smoking on the oral microflora. Of those that appear to satisfy these requirements, some early studies tended to show no differences. However, there are now a number of studies that suggest a trend for smokers to harbour more or greater numbers of potential periodontal pathogens than non-smokers without increasing the amount of plaque. This undoubtedly supports the attractive hypothesis that a significantly different subgingival environment in smokers, related to an altered immune response, should result in a different microflora. Further investigation with the latest methods is still required to confirm that such differences are directly related to smoking.

Effect of Smoking on the Periodontal Tissues

Effect of smoking on gingival blood flow

There is little or no evidence that smoking induces gingival vasoconstriction. Early studies of ANUG recognized that many affected individuals were smokers (Pindborg 1947) and it was hypothesized that the necrotic lesion may have been caused by vasoconstriction induced by nicotine and stress (Kardachi & Clarke 1974). This hypothesis was implicated in periodontitis, and a subsequent paper by Clarke et al. (1981) is often cited as evidence. In their experiment, they attempted to indirectly measure blood flow by measuring temperature from a thermistor inserted into the gingival sulcus of a rabbit. The rabbit was subjected to 10 intra-arterial infusions of nicotine into the carotid at 30 min. intervals. They reported that the gingival blood flow increased immediately following the infusion, but that recovery fell below baseline levels. There was a gradual decline in thermistor voltage to the 7th infusion but there was a gradual increase thereafter. The reported values were not related to temperature or blood flow and were not analysed statistically.

Baab & Öberg (1987) were the first researchers to question the vasoconstrictive action of nicotine (from cigarette smoking) on gingival tissues. In a Laser Doppler Flow (LDF) study of 12 young regular smokers, they showed that gingival blood flow rose by about 25% during smoking, was maintained for 5 min. and then gradually declined to baseline values. This was associated with an increase in heart rate and systolic and diastolic blood pressure. They confirmed that the blood flow to the skin of the forearm did decrease slightly, demonstrating the differences in response between peripheral skin responses and those in the head and neck. It was interesting to note that 3 of their subjects felt light headed after smoking, suggesting that the inhalation dose was greater than they normally experienced.

The study of Meekin et al. (2000) added further information to this response. They compared the response to smoking a single cigarette in a group of light/occasional smokers and heavier habitual smokers. The changes in gingival blood were not statistically significant. However. they showed quite dramatic differences in responses of LDF in the skin of the forehead. The light smokers responded with a significant increase in blood flow, paralleling the changes observed by Baab & Öberg (1987) but the heavy smokers showed no response, indicating a high level of tolerance. The increase in blood flow to the gingiva and forehead skin following an episode of smoking in 13 casual consumers of tobacco was confirmed by Mavropoulos et al. (2003). The same group had previously reported an increase in blood flow to these tissues following the topical application of tobacco snuff to the vestibular sulcus (Mavropoulos et al. 2001). Blood pressure and heart rate increased and blood flow to the thumb decreased, confirming the systemic effect of the topically absorbed nicotine. This is in contrast to cigarette smoking, where the effects are mediated by systemic absorption from the lung.

There has also been a study where Laser Doppler blood flow has been recorded in subjects who quit smoking. Morozumi et al. (2004) examined 11 periodontally healthy regular smokers who successfully quit smoking as verified by serum cotinine analysis. These researchers managed to improve the reproducibility of LDF recordings to allow measurement at time intervals over an 8-week period. They showed that the gingival blood flow had significantly increased at 3 days following quitting and that further small increases occurred up to 4 and 8 weeks. This study provides important information on the recovery of healthy gingival tissue post-quitting.

Oxygen tension in the gingival tissues

Tobacco smoke contains carbon monoxide, which is detectable in the breath of smokers and can be used to assess compliance in quit-smoking programmes (Scott et al. 2001). Oxygen saturation of haemoglobin is affected and attempts have been made to measure this in the gingival tissue of smokers and non-smokers. Hanioka et al. (2000b) showed variable results. In healthy gingiva, smokers did appear to have lower oxygen saturation, determined using tissue reflectance spectrophotometry, whereas in the presence of inflammation, the converse was shown. The same group of workers (Hanioka et al. 2000a) also examined the oxygen tension in the pockets of 34 non-smokers and 27 heavy smokers with mild to moderate periodontitis. They showed that the pocket oxygen tension was significantly lower in smokers (mean

21.9 mm Hg CI 18.1–25.7) compared with non-smokers (mean 33.4 mm Hg CI 30.5–36.31 [$p < 0.0001$]). This could have an impact on the pocket microflora (see ''The Effect of Smoking on Plaque'').

Gingival inflammation and bleeding

Some early studies suggested that smokers experienced less gingival bleeding than non-smokers (Bergstrom & Floderus-Myrhed 1983). This observation was confirmed in a comparative study of 10 heavy smokers (at least 20 cigarettes per day) and 10 non-smokers who had similar levels of periodontitis (Preber & Bergstrom 1985). These authors cited the potential vasoconstrictive effect of nicotine previously reported by Clarke et al. (1981).

The reduced bleeding on probing was further demonstrated in a study by Bergstrom & Bostrom (2001). Gingival bleeding was lower in 130 smokers (median [interquartile range, IQR] bleeding score 19.0 [13.0]) than 113 non-smokers (median [IQR] bleeding score 32.0 [20.3]), with similar levels of periodontitis ($p < 0.001$). They were also able to show a dose–response effect, which was confirmed in a much larger study of 12,385 general population subjects from the National Health and Nutrition Examination Survey III by Dietrich et al. (2004).

This reduced response in smokers has also been elegantly shown in studies using the experimental gingivitis model. In the first of these studies involving healthy dental students, 10 smokers (10–20 cigarettes per day for at least 4 years) and 10 non-smokers abstained from cleaning their mandibular anterior teeth for 28 days (Preber & Bergstrom 1986). Plaque accumulation did not differ between the groups, and although bleeding scores significantly increased in the non-smokers, there was no comparable increase in the smokers. The development of gingival redness and the volume of gingival crevicular fluid (GCF) were also lower in smokers, suggesting a suppression of the normal inflammatory response to plaque. In a follow-up study of experimental gingivitis, Bergstrom et al. (1988) evaluated the number of visible gingival vessels. There was a tendency for fewer gingival vessels at baseline in the smokers and after 14, and 28 days of plaque accumulation, the smokers had approximately half the number of visible vessels compared with the non-smokers ($p < 0.05$), despite having similar levels of plaque ($p > 0.05$). The reduced inflammatory response in this model has also been confirmed by Danielsen et al. (1990) and Lie et al. (1998a). The latter group also showed that a reduced bleeding score (approximately 50%) in smokers was apparent on both probing to the bottom of the pocket and probing the marginal gingivae.

The effect of smoking on gingival bleeding has also been shown in subjects on a quit-smoking programme. Nair et al. (2003) followed 27 subjects for 4–6 weeks during a verified successful period of quitting smoking. Gingival bleeding assessed with a constant force probe doubled (from 16% to 32%, $p < 0.001$) during this period, despite some improvement in subjects' plaque control. This demonstrates a relatively rapid recovery of the inflammatory response and corresponds with reported changes in some other parameters, such as the reduction in serum soluble intercellular adhesion molecule (sICAM) levels (Palmer et al. 2002).

Effect on the gingival vasculature

The vasculature has also been examined in histological and immunocytochemical studies. In a very limited study of one histological section from three smokers and four non-smokers, Mirbod et al. (2001) found that there were a high proportion of small vessels compared with large vessels in smokers compared with non-smokers, but no difference in the vascular density. The region chosen for study was the connective tissue beneath the external gingival epithelium, which was therefore remote from the pocket wall/sulcus and the inflammatory lesion. Sönmez et al. (2003) did not show differences in the density or number of Factor VIII-labelled vessels in gingival tissues obtained at the time of periodontal surgery from 38 smokers and 36 non-smokers. The orientation and location of the specimens were not described.

A more comprehensive histological comparison of smokers and non-smokers was presented by Rezavandi et al. (2002), who labelled the vessels by immunocytochemical staining to the von Willebrand factor, ICAM-1 and E-Selectin. They reported that a significantly larger number of vessels were observed in inflamed tissues of non-smokers than smokers ($p < 0.05$). In addition, the proportion of the total number of vessels expressing ICAM-1 in non-inflamed sites was greater in non-smokers compared with smokers ($p < 0.05$). This suggested that the inflammatory response in smokers with periodontitis may not be accompanied by an equivalent increase in vascularity, and that reduction in endothelial ICAM-1 expression could affect neutrophil emigration from the vessels (plus see ''Evidence from Studies on GCF''). Moughal et al. (1992) described the expression of ICAM-1 and E-Selectin (Endothelial cell leukocyte adhesion molecule-1) in the blood vessels of the gingival connective tissue and strong expression of ICAM-1 in the junctional and sulcular epithelium. The expression of these molecules was similar in both healthy and inflamed tissue of just six subjects of undisclosed smoking status participating in an experimental gingivitis study. Also, in an earlier study comparing lesions of periodontitis and gingivitis in 21 subjects, Gemmell et al. (1994) did not find any differences in the expression of adhesion molecules on endothelial cells (or lymphocytes), but there was no account of smoking status.

Conclusions

Smoking has a long-term chronic effect, impairing the vasculature of the periodontal tissues rather than a simple vasoconstrictive effect following a smoking episode. The suppressive effect on the vasculature can be observed through less gingival redness, lower bleeding on probing and fewer vessels visible clinically and histologically. This may also have relevance to the healing response with impairment of revascularization.

Smoking and Neutrophil Function

Tobacco smoke exposure increases the number of neutrophils found in the systemic circulation (van Eeden & Hogg 2000, Iho et al. 2003, Sorensen et al. 2004) and those exiting the pulmonary microvasculature into the lungs (Chalmers et al. 2001, Seagrave et al. 2004). Despite inducing a significant systemic neutrophilia, tobacco smoking does not seem to affect the numbers of neutrophils entering the gingival sulcus and oral cavity. Indeed, limited evidence suggests that the numbers of neutrophils reaching the gingival sulcus in smokers may even be reduced (Eichel & Shahrik 1969, Pauletto et al. 2000). These findings imply that neutrophil

transmigration across the periodontal microvasculature is impeded in tobacco smokers. It may be relevant to note that neutrophils are known to be sequestered in the pulmonary microvasculature and are hypothesized to contribute to the degradation of lung tissues while trapped within this vascular compartment (MacNee et al. 1989, Terashima et al. 1999). Neutrophils are primary mediators of protection against periodontal plaque bacteria (Dennison & Van Dyke 1997). However, neutrophil bacteriocidal activity involves multiple non-specific mechanisms, and the inappropriate and/or continuous activation of periodontal neutrophils is thought to contribute to the degradation of gingival tissues and the progression of inflammatory periodontal disease (Deas et al. 2003).

Neutrophils express functional receptors for several components and metabolites of tobacco smoke, such as nicotine, cotinine (primarily the $\alpha 3\ \beta 4$ subtype of nicotinic receptors; Benhammou et al. 2000) and aryl hydrocarbons (Ackermann et al. 1989). The numbers of nicotinic receptors expressed by human neutrophils are increased in smokers and decline on cessation (Lebargy et al. 1996).

Neutrophils also express several receptors for endogenous factors such as interleukin-8 (IL-8) (Zeilhofer & Schorr 2000), ICAM-1 (Selby et al. 1992, Scott et al. 2000) and tumour necrosis factor-α (TNF-α) (Akgul & Edwards 2003), whose natural agonists have been reported to be dysregulated in tobacco smokers in the periodontal environment and elsewhere [(IL-8: Fredriksson et al. 2002, Iho et al. 2003); ICAM-1 (Fraser et al. 2001; Palmer et al. 2002, Rezavandi et al. 2002); and TNF-α (Fredriksson et al. 2002)].

Thus, multiple receptor-agonist couples have been identified, suggesting that tobacco smoke is capable of affecting neutrophil function both directly and indirectly. For example, circulating soluble circulating soluble ICAM-1 is a physiologically active, immunomodulatory molecule whose concentrations increase in a predictable dose-dependent manner in tobacco smokers and rapidly return to baseline on smoking cessation (Palmer et al. 2002). The major natural ligands for ICAM-1 are the β_2-integrin complexes, which are expressed by human neutrophils and other leucocytes. The importance of the engagement of β_2-integrin complexes, which stimulates elastase and matrix metalloproteinase (MMP) release, in neutrophil-mediated tissue injury and degradation is established in several disease entitities (Barnett et al. 1998, Scott & Palmer 2002).

Neutrophil-derived degradative proteases in tobacco smokers

It has long been hypothesized that tobacco smoking increases the proteolytic activity of neutrophils (Donaldson et al. 1991). The critical importance of neutrophil recruitment and neutrophil-derived proteolytic enzymes, particularly MMPs and elastase, to the tobacco-induced destruction of pulmonary vessels and tissues is well established (Wright et al. 2003, Churg et al. 2004). Tobacco smoking leads to significant increases in the circulating burden of neutrophil elastase and MMPs in humans (Nakamura et al. 1998, van Eeden & Hogg 2000). Tobacco smoking has also been shown in human skin to decrease the rate of synthesis of specific collagen types, by increasing the production of collagen-degrading enzymes, and decreasing levels of the major endogenous MMP inhibitor, tissue inhibitors of MMP-1 (Knuutinen et al. 2002).

While the tobacco-induced release of proteolytic enzymes from neutrophils has not been demonstrated definitively in the periodontal tissues themselves, neutrophils are considered to be a major source of the elastase and MMPs associated with periodontal disease destruction (Soder et al. 2002, Persson et al. 2003). Furthermore, tobacco smoke and components are known to stimulate the release of such enzymes from neutrophils in vivo and in vitro (Seow et al. 1994, Seagrave et al. 2004). Seow et al. (1994) examined the effects of nicotine on neutrophil function at concentrations achievable in oral tissues and showed an enhancement of neutrophil degranulation.

It is, therefore, entirely possible that tobacco may contribute to the progression of periodontal disease at least in part through the induction of protease release from periodontal neutrophils.

Neutrophil respiratory burst

The respiratory burst represents the combined oxygen-dependent processes by which neutrophils kill phagocytosed bacterial cells through the generation of multiple reactive oxygen and reactive nitrogen species. A compromised respiratory burst may reduce the capacity of neutrophils to destroy plaque bacteria. Several studies have suggested that cigarette smoke constituents may inhibit the respiratory burst of neutrophils (Drost et al. 1992, Pabst et al. 1995, Sorensen et al. 2004). However, such studies utilize different media for neutrophil suspension/challenge, with and without cations and glucose, which have a profound effect on the respiratory burst. In the Sorensen et al. (2004) study, a decrease in luminol-dependent enhanced chemiluminescence (ECL) was demonstrated in smokers. However, luminol ECL represents total luminescence and does not differentiate extracellular from intracellular radical release. Furthermore, luminol is regarded as a reporter molecule for hypochlorous acid production and thus reflects myeloperoxidase activity, rather than directly measuring superoxide anion release. Lucigenin is the substrate most commonly used to measure superoxide release from NADPH-oxidase activity (Koie et al. 2001). Therefore, results of studies on neutrophil function in relation to smoking status require careful interpretation.

Nguyen et al. (2001) have recently demonstrated that gas-phase cigarette smoke may lead to a suppression of neutrophil NADPH oxidase, but not myeloperoxidase activity, that is at least partially mediated by volatile reactive α,β-unsaturated aldehydes, such as acrolein. Other researchers have confirmed suppression of the oxidative burst in neutrophils treated with aqueous-phase smoke extract, coupled with a significant hindrance of phagocytotic ability (Zappacosta et al. 2001).

However, other studies appear to show that tobacco constituents can exacerbate aspects of the respiratory burst in human neutrophils. Iho et al. (2003) suggest that nicotine can increase IL-8 production by neutrophils (which would be expected to recruit further neutrophils) and enhance the production of reactive species, particularly $ONOO^-$. Additionally, the work of Gillespie et al. (1987) showed a potentiated release of superoxide from neutrophils in rats exposed to either cigarette smoke or to i.p. -injected nicotine.

On balance, the literature would suggest that smoking affects the NADPH-oxidase respiratory burst, but, because of differences in neutrophil handling and reporter substrates, it is not possible to conclude whether NADPH-oxidase responses are enhanced or suppressed.

Furthermore, many of the studies do not examine the activity of the NADPH-oxidase in smoker and non-smoker patient neutrophils simultaneously. This is vital for assay consistency because ex-vivo neutrophil responsiveness varies widely from day to day.

Neutrophil migration and chemotaxis

Early studies suggested detrimental effects of tobacco smoke extract on migration of oral neutrophils (Eichel & Shahrik 1969, Kraal et al. 1977). The actin cytoskeleton is critical in facilitating neutrophil motility that is required for extravasation across the periodontal microvasculature and subsequent migration of neutrophils towards inflammatory stimuli. Recently, Ryder et al. (2002) have shown that tobacco smoke exposure may impair f-actin kinetics. This is in keeping with the earlier data of Drost et al. (1992), who observed that smoke-exposed neutrophils exhibited reduced deformability, an effect mediated through the actin component of the cytoskeleton. This reduced neutrophil deformability was accompanied by a compromised ability to traverse micropore membranes similar in dimension to capillaries.

Seow et al. (1994) examined the effects of nicotine on neutrophil function at concentrations achievable in oral tissues. The results showed a dose-dependent suppression of both chemotaxis and phagocytosis. Earlier, Bridges & Hsieh (1986) had fractionated cigarette smoke condensate (CSC) and demonstrated that the more polar fractions were potent inhibitors of chemotaxis, while the fractions containing nicotine and those containing polycyclic hydrocarbons were weak inhibitors of neutrophil chemotaxis. Another group has shown that neutrophil cell spreading and chemokinesis, but not chemotaxis itself, are impaired following in vitro smoke exposure (Selby et al. 1992). While MacFarlane et al. (1992) could not demonstrate tobacco-induced chemotactic defects in neutrophils from smokers with refractory periodontitis, impaired phagocytosis was evident in neutrophils from these subjects.

Nowak et al. (1990) observed that the influence of nicotine on neutrophil chemotaxis was dependent on the stimulating dose, with low concentrations of nicotine stimulating neutrophil chemotactic responsiveness to fMLP and high nicotine concentrations being inhibitory. Similarly, Gillespie et al. (1987) found that i.p. injection with 0.2 mg/kg nicotine reduced fMLP-induced neutrophil chemotaxis, in contrast to the potentiating actions of a lower dose (0.02 mg/kg nicotine). In an ex vivo study, Sorensen et al. (2004) demonstrated increased chemotaxis in the peripheral blood neutrophils of smokers, but this decreased again when the same subjects quit smoking.

When studying chemotaxis in neutrophils, it should be kept in mind that neutrophils respond to multiple chemotactic stimuli, the mechanisms of all, none, or some which may be affected by tobacco smoke exposure. Therefore, variable results are to be expected, depending on the experimental system used.

Neutrophil priming (hyper-reactivity)

Koethe et al. (2000) have suggested that, in vitro, components of tobacco smoke that are present in CSC can have a profound influence on neutrophil effector function. CSC induced a greater than twofold increase in fMLP receptor expression on the neutrophil surface, rendering the cells ''primed'', resulting in a twofold increase in the release of both superoxide and elastase on subsequent stimulation with fMLP.

Tobacco-induced increases in fMLP receptor numbers have been noted by others (Matheson et al. 2003). In the periodontal environment, where there is a plentiful source of fMLP, priming of gingival neutrophils by tobacco smoke components could lead to an increase in the local burden of potentially degradative elastase and superoxide in response to the plaque bacteria – a hyperinflammatory response. In further support of this hypothesis, Gustafsson et al. (2000) have shown that the priming capacity of TNF-α, measured as generation of oxygen radicals from stimulated neutrophils, is higher in neutrophils from smokers compared with neutrophils from non-smokers. The same authors also showed that smoking potentiated the generation of radicals in individuals with periodontitis.

Conclusions

Neutrophils are critical cells in the maintenance of periodontal health because of their multifaceted roles in the control of plaque bacteria, but they may also contribute to the progression of periodontitis in chronic inflammatory responses. While there are conflicting data, it is clear that tobacco smoking affects multiple functions of neutrophils and may shift the net balance of neutrophil activities into the more destructive direction.

Smoking and Lymphocyte Function

The immune system of mammals has evolved to protect the host. Innate and adaptive immunity operate together, and there are innate immune mechanisms that help to focus and facilitate adaptive immune responses. In order to protect the host, the adaptive immune system must be able to recognize antigens and then mount an appropriate response. Antigens are recognized by lymphocytes. T lymphocytes recognize antigens presented in association with human lymphocyte antigens by antigen-presenting cells such as dendritic cells, macrophages and B cells. Following antigen presentation and appropriate costimulation, effector cells are formed. This process involves clonal selection of the T cell clones bearing T cell receptors that can recognize epitopes on antigen, and clonal proliferation, thereby ensuring there are sufficient numbers of the appropriate T cells available to combat infection and protect the host. In the effector phase, activated T cells differentiate into cytotoxic T cells, and T helper cells. Cytotoxic T cells express CD8 molecules on their cell surface with T cell receptor (TCR), while T helper cells typically express CD4 in association with TCR and have either a T helper cell (Th1) or pro-inflammatory phenotype activating macrophages and stimulating cytotoxicity, whereas T helper 2 (Th2) cells are essential for successful antibody responses as they control B cell growth and differentiation, as well as immunoglobulin class switching.

T lymphocytes

There are many studies that show leucocytosis in smokers (Corre et al. 1971, Hughes et al. 1985). Most studies have examined T cell subsets and report different findings: reduced, increased or no change in the number of CD4 T cells (Ginns et al. 1982, Smart et al. 1986, Johnson et al. 1990, Loos et al. 2004). Smokers suffer from respiratory infections more frequently than non-smokers. This finding is often interpreted to mean the effects of smoking on immunity are localized to the lung (Finklea et al. 1971), and this is further

supported by finding little correlation between alterations in T cell numbers in bronchial alveolar lavage fluid (BALF) and peripheral blood in smokers. Furthermore, the total number of lymphocytes is increased in BALF and the CD4 cell subpopulation is reduced (Costabel et al. 1986, Wallace et al. 1994), producing a reduced CD4/CD8 ratio in smokers compared with non-smokers, while the CD4/CD8 ratio in peripheral blood is similar (Ancochea et al. 1993, Wallace et al. 1994). On cessation of smoking, the number of CD4 cells in peripheral blood returns to levels observed in people who have never smoked (Ginns et al. 1982, Loos et al. 2004).

More recently, Loos et al. (2004) examined 112 adults, 76 with periodontitis and 36 control subjects. Subjects were classified into non-smokers (those who quit within the last 10 years), light smokers (those who smoked <10 cigarettes/day) or heavy smokers ($\geqslant 10$ cigarettes/day). The total leucocyte count was highest among the heavy smokers and significantly higher compared with non-smokers irrespective of periodontal disease status, and was reflected in increased neutrophil numbers but not in lymphocyte or monocyte numbers. Although there was a similar trend towards elevated neutrophil counts in the controls, moderate and severe periodontitis, this was not statistically significant. However, smoking status acted as a significant covariate for total leucocytes, neutrophils, lymphocytes and monocytes. Analysis of CD3 T cells, CD4 and CD8 T cell subsets, and CD4/CD8 ratios revealed no significant differences between controls and periodontitis groups. However, leucocyte counts are known to vary according to ethnic group (Tollerud et al. 1989, 1991), and in this study it is not clear how well the subjects were matched for race, as they were only grouped as either Caucasian or non-Caucasian (Loos et al. 2004).

Animal studies have indicated that chronic exposure of rats to the vapour phase of cigarette smoke does not lead to significant changes in immune responses. It is therefore likely that the particulate phase of cigarette smoke confers the immunosuppressive properties (Hoffmann 1979, Hoffmann & Wynder 1986). Unfortunately, among the numerous possible compounds present, the particulate phase of cigarette smoke consists of nicotine polycyclic aromatic hydrocarbons, tobacco glycoprotein and some metals (Hoffmann & Wynder 1986). Each compound has its own effects and, while nicotine, benzo(a)pyrene and benzo(a)anthracene (polycyclic aromatic hydrocarbons) are immunosuppressive (Geng et al. 1995, 1996), tobacco glycoprotein and metals present in cigarette smoke are immunostimulatory (Francus et al. 1988, 1992, Brooks et al. 1990). Furthermore, acute or chronic exposure to hydrocarbons may stimulate (Schnizlein et al. 1982) or inhibit (White 1986) the immune response. Thus, the net effect is dependent upon the dose and duration of the exposure to the components of tobacco smoke (Tollerud et al. 1991).

T cell function

The effects of tobacco smoking on T cell function/proliferation are controversial. Some studies show significant reductions in T cell proliferation compared with controls when lymphocytes are stimulated with mitogens, while others show no significant differences between lymphoproliferative responses of smokers and non-smokers (Sopori et al. 1994). Tobacco smoke has been shown to reduce the proliferative response of both peripheral blood and BALF lymphocytes to both T cell mitogens and antigens (Chang et al. 1990, Johnson et al. 1990). Nicotine can inhibit T cell proliferation to mitogens in in vitro experiments using peripheral blood from non-smokers and may even induce T cells, which can suppress T cell function (Petro et al. 1992). It is difficult to duplicate human smoking habits in animals that are often only exposed to cigarette smoke for just a few minutes and not years, as is often the case in humans. When animals are exposed to tobacco smoke, antibody responses have also been found to be inhibited in lung-associated lymph nodes, but not in extra-pulmonary lymph nodes (Sopori et al. 1989), suggesting that the immune response is altered locally. Chang et al. (1990) showed that antigen-specific T cell proliferation was inhibited following smoke inhalation in bronchial nodes draining the lung, but was unaffected by tobacco smoke in distant lymph nodes.

Recently, Loos et al. (2004) investigated the proliferative capacity of T cells in whole blood lymphocyte culture assays. No significant differences were found between the control subjects, periodontitis patients and smoking status following stimulation with phytohaemagglutinin (PHA) or Mab to CD3 and CD28. As smoking status correlates with the numbers of lymphocytes, the use of whole-blood culture assays would not allow distinction between impaired/enhanced T cell responses or impaired/enhanced monocyte responses (Loos et al. 2004).

B cells and immunoglobulins

B cells recognize antigen once it has bound to antigen-binding sites of immunoglobulin on the B cell antigen receptor, namely antibody expressed on the B cell surface. In order to mount successful humoral immune responses, B cells require T helper cell-derived cytokines to proliferate and differentiate into plasma cells (as well as for immunoglobulin class switching).

In experiments in animals and humans, tobacco smoke has been found to affect both humoral immunity and cell-mediated immunity (Sopori et al. 1994, Sopori & Kozak 1998). Chronic exposure of rats to nicotine inhibits antibody-forming cell responses and this immunosuppression appears to be the result of impairment of antigen-mediated T cell signalling (Geng et al. 1996, Sopori et al. 1998). These findings are supported by reports indicating that serum IgG levels are reduced in smokers (Gulsvik & Fagerhol 1979, McSharry et al. 1985, Quinn et al. 1996, 1998) and in periodontitis patients; non-smokers have higher levels of IgG2 compared with smokers (Graswinkel et al. 2004).

The effects of cigarette smoking on serum IgA and IgM classes are controversial, with some reports indicating suppression of IgM and IgA levels (Gulsvik & Fagerhol 1979), while other studies indicate no effect of smoking on either class of antibody (Andersen et al. 1982, McSharry et al. 1985, Graswinkel et al. 2004). IgE is greatly elevated in smokers, and not related to enhanced skin reactivity (Burrows et al. 1981, 1982).

There are some researchers who found no suppression of total IgG concentrations in smokers (Merrill et al. 1985, O'Keeffe et al. 1991, Graswinkel et al. 2004). These conflicting findings may reflect differences in the populations studied. O'Keeffe et al. (1991) examined an older population with a mean age of 60 years (50–80) in whom IgG2 levels tend to be higher. In addition, in order to determine whether IgG subclasses vary in different populations,

the total IgG needs to be determined and related to IgG subclasses. However, this analysis is not always carried out. Studies of IgG subclasses in smokers and non-smokers with periodontal disease show that IgG2 is depressed in smokers. However, serum IgG2 levels in African American subjects are unaffected by smoking, except in generalized early-onset periodontitis (Quinn et al. 1996). When Quinn et al. (1998) investigated black subjects, they found a reduction in IgG1 and IgG4 in chronic periodontitis.

Further evidence that serum immunoglobulin subclass concentrations are strongly influenced by periodontal disease status and race have been provided by Gunsolley et al. (1997) and Lu et al. (1993, 1994). Localized juvenile periodontitis patients have elevated levels of IgG2 compared with other race-matched and periodontal disease groups. In addition, black patients show increased concentrations of all IgG subclasses compared with white subjects with the same periodontal diagnosis (Lu et al. 1993, 1994). There is clearly a complex relationship between immunoglobulin subclass, race, age, periodontal diagnosis and smoking.

B cell function

B cell numbers are similar in smokers and non-smokers (Sopori et al. 1989). In peripheral blood, there is a decrease in proliferative response to polyclonal B cell activators (B cell mitogens) and antigens (Sopori et al. 1989), suggesting that B cell function is impaired in smokers. On cessation of smoking, B cell function returns to normal (Mili et al. 1991, Reynolds et al. 1991).

Sopori et al. (1989) showed that in smoke-exposed animals, there is a reduction in antigen-induced proliferation. This can first be detected in lymphoid tissue with prolonged exposure to tobacco smoke, suggesting that B cell function may be affected by combustion by-products of tobacco smoke. Tobacco glycoprotein (a polyphenolic protein in tobacco) is a potent B cell mitogen (Francus et al. 1988) and stimulates production of immunoglobulin classes (IgM, IgG and IgA). In vitro studies in which smokeless tobacco was used to stimulate murine splenic and mesenteric lymph node cells indicated that this form of tobacco could induce the production of polyclonal IgM (Hockertz et al. 1994). Therefore, although there are B cell mitogens in tobacco, the observed effect is determined by the net immunostimulatory/immunosuppressive properties of tobacco and its combustion by-products.

Natural killer (NK) cells

NK cells, components of the innate immune system, are large granular non-T non-B lymphocyte-like cells that make up a small proportion of peripheral blood lymphoid cells. Unlike T and B cells, NK cells do not have antigen-specific receptors, but are able to recognize and kill antibody-coated target cells (antibody-dependent cellular cytotoxicity) and this is triggered when antibody bound to the surface of the cell interacts with Fc receptors on the NK cells. The mechanism of attack is analogous to cytotoxic T cells involving the release of granules containing perforins and granzymes. NK cells also produce chemokines and inflammatory cytokines such as interferon-γ and TNF-α.

Reduced NK cytolytic activity has been reported in smokers (Ferson et al. 1979, Tollerud et al. 1989). NK cells from BALF of smokers show reduced cytolytic activity compared with BALF NK cells of non-smokers (Takeuchi et al. 1988). Some workers have found no differences between NK cells in smokers and non-smokers (Hughes et al. 1985). Others have found that the effect of smoking on NK cells is reversible and cytolytic activity may increase even within a short period of a month of smoking cessation (Meliska et al. 1995). This contrasts with the findings of Tollerud et al. (1989), who found that the numbers of NK cells in peripheral blood do not recover on cessation of smoking. These discrepancies may, in part, reflect differences in methodology, identification and determination of NK cell numbers and NK cell cytotoxicity.

In Caucasian subjects, there is a significant decrease in the proportion of circulating NK cells in smokers that may persist for some time after the cessation of smoking. Tollerud et al. (1989) found that in contrast to Caucasian smokers, African-American smokers had numbers of NK cells similar to non-smoking African Americans. While this may at first appear surprising, there are a number of reported differences between ethnic groups in terms of white blood cell counts, numbers of T and B cells and immunoglobulin levels (Tollerud et al. 1989, 1991,1994).

Conclusions

There are inconsistencies and variations in findings reflecting the complex relationship between smoking, race, periodontal diagnosis and age. Most studies have focused on peripheral effects, mainly on the lung, with little periodontal-specific research. Furthermore in many periodontal diseases, the important antigens are not known and it is therefore difficult to assess the effect of smoking on antigen-specific responses that are relevant to periodontitis.

Evidence from Studies on GCF

GCF has been the subject of intense research in periodontology and readily lends itself to comparative studies of various conditions. However, the reliability of data is sometimes problematic because of the difficulty in measuring and assaying the small volumes obtained in many cases and the variations in collection protocols (different collection strips, times and numbers of samples) and processing methodology. Some studies express GCF concentrations and some ''total amounts'' of a substance per unit sample time. The latter has been recommended in studies that attempt to identify markers of active disease (Lamster et al. 1986, Chapple et al. 1999). However, for studies that address pathogenesis, it is vital to measure GCF volumes and examine concentrations as well as total amounts of the analtye under investigation. This is because studies have shown a significant relationship between pocket depth and GCF volume and the latter is a major confounder of analyte concentration in association studies of periodontitis (Brock et al. 2004). Finally, the comparative contribution of serum- and tissue-derived products in a GCF sample is impossible to determine.

Smoking may result in lower resting GCF flow rate (Persson et al. 1999); the increase in GCF during an experimental gingivitis may be less in smokers (Bergstrom & Preber, 1986), and an episode of smoking may produce a transient increase in GCF flow rate (McLaughlin et al. 1993). In a study examining subjects on a quit-smoking programme, Morozumi et al. (2004) showed that GCF flow was greater at 5 days post-quitting. These apparently contradictory findings can be related to the acute and chronic effects of smoking on blood flow (see ''Effect of smoking on gingi-

val blood flow'') and the inflammatory response (see ''Gingival inflammation and bleeding'').

Cytokines

Bostrom et al. (1998) showed higher levels of TNF-α in GCF in smokers ($n = 30$) and former smokers ($n = 19$) compared with non-smokers (n = 29), with comparable levels of moderate/severe periodontitis. The smokers had significantly less bleeding on probing, and the GCF samples were collected using a washing technique and therefore volumes were not accurately determined (this was compensated by using measurement of serum albumin). No attempt was made to compare GCF levels with those found in the serum. In a follow-up study of a comparable group of subjects using similar protocols, Bostrom et al. (1999) confirmed the presence of higher levels of TNF-α in a smaller group of smokers. However, no differences were found in the levels of IL-6, which were frequently below the detection levels of their ELISA. The same research group (Bostrom et al. 2000) compared levels of IL-1β and IL-1ra in pooled GCF washing samples in 22 smokers and 18 non-smokers. No significant differences were found. However, Rawlinson et al. (2003) found levels of IL-1β and IL-1ra to be significantly lower in GCF from diseased sites in smokers compared with non-smokers, using a 3 min. paper strip sample measured with a Periotron 6000 and collected following the discarding of a 30 s sample.

Petropoulos et al. (2004) showed that the concentration of IL-1α in GCF of smokers was approximately half that found in non-smokers ($p < 0.01$), which supported their previous observations (Shirodaria et al. 2000) and confirmed a wide site-to-site variation. This group did not show differences in polymorphonuclear cell (PMN) numbers between smokers and non-smokers eluted from Durapore collection strips left in place for 10 s. In a 10-day experimental gingivitis study, Giannopoulou et al. (2003) claimed higher levels of IL-8 and lower levels of IL-4 in smokers GCF at baseline and the end of the period. However, they did not measure GCF volume and reported the total amount per 20 s sample.

Other factors

Pauletto et al. (2000) did not directly measure GCF levels but examined saliva and oral rinse samples for elastase activity. Elastase activity was lower in the smokers, as were neutrophil counts. Alavi et al. (1995) showed significantly lower concentrations of GCF elastase in smokers compared with non-smokers with similar levels of disease and GCF volumes (functional elastase: smokers 30.21 ± 17.6 ng/μl, non-smokers 73.77 ± 75.26 ng/μl, $p < 0.05$ and elastase complexed with α1-antitrypsin smokers 8.97 ± 6.54 ng/μl, non-smokers 25.71 ± 22.07 ng/μl, $p < 0.001$). Although PMN numbers were reduced in smokers' GCF, this was not significant. Significantly lower concentrations of α-2-macroglobulin and total amounts of α-1-anti-trypsin have been reported in heavy smokers with moderate to severe periodontitis by Persson et al. (2001).

Molé et al. (1998) examined levels of sICAM-1 in GCF (paper strip for 2 min.) and showed higher levels at sites of inflammation, but smoking status was not taken into account. Following earlier reports of higher levels of sICAM-1 in the serum of smokers (Koundouros et al. 1996) regardless of their periodontal status, Fraser et al. (2001) examined levels of this molecule in the GCF of smokers and non-smokers. They confirmed higher systemic serum levels of sICAM-1 (smokers 331 ng/ml, non-smokers 238 ng/ml; $p = 0.008$) but, in contrast, levels in GCF were significantly lower in smokers (smokers 83 ng/ml, non-smokers 212 ng/ml; $p = 0.013$). The smokers had significantly lower sICAM-1 levels in GCF compared with serum (in contrast to the non-smokers), although levels of cotinine were comparable (which would indicate that this molecule is unaffected in transit from the circulation to the periodontal pocket).

Conclusions

It would seem logical to expect that factors that are associated with tissue destruction should be higher in smokers than non-smokers. This is blatantly not the case for many of these factors when assayed in the GCF. For the most part, research has demonstrated that there are lower levels of cytokines, enzymes and possibly PMNs. This correlates with the lower levels of inflammation observed clinically and within the tissues. The GCF could be viewed as an end product of the destructive process, and lower levels of factors may simply indicate higher levels of activity within the tissue or effects on gingival fluid flow dynamics.

Smoking and fibroblast function

The effects of nicotine and some other components of tobacco smoke have been tested in in vitro studies on gingival and periodontal ligament (PDL) fibroblast lines. All published studies, with the exception of the study of Peacock et al. (1993), have demonstrated detrimental effects, which could have significant impact in the inflammatory destructive process and the healing response. However, most of these studies used concentrations of nicotine and cotinine that were far higher than those expected in plasma of smokers (nicotine 5–50 ng/ml, 0.03–0.3 μmol/l; cotinine 50–500 ng/ml, 280–2800 nmol/l), and the concentrations used by some are difficult to verify because of lack of information. In an early descriptive study, Raulin et al. (1988) suggested that cell orientation and attachment of human foreskin fibroblasts to glass or dentine surfaces were affected by nicotine at concentrations comparable with plasma levels (25–50 ng/ml), with more striking changes at higher concentrations (200–400 ng/ml). Nicotine at comparable levels was found on root surfaces of periodontally affected teeth extracted from smokers, and the levels could be reduced following root planing (Cuff et al. 1989).

Gingival fibroblasts

Hanes et al. (1991) showed that gingival fibroblasts bound nicotine, which was subsequently taken up and then released into an in vitro culture system. They did not demonstrate the presence of specific receptor binding for nicotine. Peacock et al. (1993) were the only researchers to show a positive effect of nicotine on the proliferation and attachment of gingival fibroblasts to plastic. They used a series of nicotine concentrations close to normal serum levels (4–64 ng/ml, assuming that the units described were μm/ml). Significant inhibition of proliferation of gingival fibroblasts at very high concentrations of nicotine of 10–75 μg/ml was demonstrated by Tipton and Dabbous (1995), who also showed reduction in the production of type 1 collagen and fibronectin and an increase in the collagenase activity in the culture media. Tanur et al. (2000) examined the effects

of nicotine on the attachment of gingival fibroblasts to glass and non-diseased root specimens. Concentrations of 25–100 ng/ml affected the orientation of cells. Higher concentrations produced cytoplasmic vacuolation and attachment to the surfaces was more easily disrupted. Inhibition of collagen production by gingival fibroblasts could also be demonstrated when they were grown in the presence of epithelial cells that had previously been exposed to 50–500 μg/ml nicotine (Giannopoulou et al. 2001). There is some evidence that gingival fibroblasts from smokers may be less susceptible to the cytotoxic effects of high levels of nicotine (Checchi et al. 1999), possibly because of the development of tolerance. Gingival fibroblasts were exposed to acrolein and acetaldehyde, present in the volatile fraction of tobacco smoke, by Cattaneo et al. (2000) and Poggi et al. (2002). These substances inhibited cell attachment and cell proliferation at concentrations of 3×10^{-5} M for acrolein and 10^{-3} M for acetaldehyde. They observed cellular changes at the light and electron microscopical levels, which included disruption of cell orientation, presence of large vacuoles and dense residual bodies in the cytoplasm. The same group showed significant reduction in cell viability and disruption to the microtubules, intermediate filaments and actin (Poggi et al. 2002).

PDL fibroblasts

PDL fibroblast growth and attachment to tissue culture plates was inhibited by nicotine at high concentrations (over 1 mg/ml) and no effects were seen at concentrations comparable with plasma levels in smokers (James et al. 1999). Cotinine tested at lower concentrations (up to 10 μg/ml) failed to demonstrate a significant effect. Vacuolation of PDL fibroblasts exposed to high concentrations of nicotine was observed by Giannopoulou et al. (1999) and significant inhibition of proliferation at concentrations of 100 ng/ml to 2 μg/ml. Nicotine at high concentrations (5–25 mM) was also shown to be cytotoxic by Chang et al. (2002). They confirmed that PDL cell proliferation was inhibited in a dose-dependent manner, as was protein synthesis. An interesting observation was the protective effect of the antioxidant 2-oxothiazolidine-4-carboxylic acid (OTZ) in their cytotoxicity assay. Gamal and Bayomy (2002) examined PDL fibroblast attachment to root surfaces that were obtained from patients 7 days after thorough root planing. Cell attachment was significantly less on root surfaces obtained from heavy smokers compared with non-smokers and healthy controls.

Conclusions

Smoking has a detrimental effect on clinical healing of non-surgical and surgical treatment modalities in periodontology (as reviewed by Kinane & Chestnutt 2000). The biological basis for this is undoubtedly multifactorial as smoking affects the vasculature and revascularization, the inflammatory response and fibroblast function, as outlined above. It is not possible to estimate the in vivo potential of these effects based upon the data from these in vitro experiments, which usually test high levels of nicotine and do not take the other noxious compounds into account. Nevertheless, it is likely that smoke products will affect fibroblast recruitment and adhesion to root surfaces.

Overall Conclusions

Tobacco smoking has widespread systemic effects, many of which may provide mechanisms for the increased susceptibility to periodontitis and the poorer response to treatment. As an environmental factor, smoking interacts with the host and the bacterial challenge. The host genetic and environmental interaction is of the utmost importance. As knowledge of the genetic susceptibility to periodontitis increases, this will provide further opportunities to explore this relationship with tobacco smoking (Kornman et al. 1997, Fraser et al. 2003, Meisel et al. 2003, Berglundh et al. 2003, Ryder et al. 2004). It is quite possible that many of the pathogenic mechanisms involved in tissue degradation in periodontitis in tobacco smokers could be quite different from those involved in non-smokers.

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Clinical Relevance

Epidemiological and clinical studies have confirmed that tobacco smoking is a major risk factor in periodontal disease. Smoking is associated with more attachment loss, bone loss and tooth loss, but, paradoxically, less signs of inflammation. Extensive clinical trials have also shown poorer responses to non-surgical and surgical treatment in smokers. The mechanisms whereby smoking affects the inflammatory and immune responses have direct relevance in periodontitis in smokers but may also reveal important information about the pathogenic mechanisms in non-smokers. Future research needs to consider the recovery of these responses in subjects who quit smoking.