Chapter 12

Modifying Factors

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Diabetes, pregnancy, and tobacco smoking have profound and far-reaching effects on the host, including effects on the:

1. Physiological response
2. Vascular system
3. Inflammatory response
4. Immune system
5. Tissue repair.

They therefore have the potential to modify the:

1. Susceptibility to disease
2. Plaque microbiota
3. Clinical presentation of periodontal disease
4. Disease progression
5. Response to treatment.

Diabetes and smoking were cited as risk factors for periodontitis in Chapter 7 and the epidemiological evidence for their association with periodontitis was dealt with. Both factors are particularly important because they may affect the individual over a great many years, usually decades, and challenge the host to varying degrees. In contrast, pregnancy is of relatively short duration (although possibly with multiple episodes) but should be considered in relation to other hormonal changes which occur at puberty, menopause, and in women on hormonal contraceptives.

These three modifying factors are extremely important in many other disease processes, for example cardiovascular disease, which also affects people to varying degrees. Much of this variation in susceptibility is probably due to genetic interactions, and there is increasing evidence of important associations with many genetic polymorphisms. There will undoubtedly be emerging genetic evidence to link periodontal disease susceptibility to modifying factors considered in this chapter.

Diabetes mellitus

Diabetes mellitus (DM) is a complex disease with varying degrees of systemic and oral complications, depending on the extent of metabolic control, presence of infection, and underlying demographic variables. This has led to conflicting results in epidemiologic studies, with regard to periodontal disease presentation in diabetic patients and their response to treatment. This section deals with diabetes and its implications on the host response to bacterial plaque, in the context of clinical and laboratory data pertaining to periodontal disease.

Type 1 and type 2 diabetes mellitus

DM is categorized as type 1 and type 2 DM. Type 1 DM develops due to impaired production of insulin, while type 2 DM is caused by deficient utilization of insulin. Type 1 DM results from destruction of the insulin-producing β cells of the pancreas. This can occur when genetically predisposed individuals succumb to an inducing event such as a viral infection or other factors that trigger a destructive autoimmune response (Szopa *et al*. 1993). Approximately

10–20% of all diabetics are insulin-dependent or type 1. They usually have a rapid onset of symptoms associated with a deficiency or total lack of insulin and the condition may be difficult to control. Nearly 90% are diagnosed before the age of 21 years.

Type 2 DM results from insulin resistance, which also contributes to cardiovascular and other metabolic disturbances (Murphy & Nolan 2000). However, insulin production may decrease later in the disease process and require supplementation (Slavkin 1997), in addition to controlling diet or using oral hypoglycemic agents. The onset of symptoms in type 2 DM is more gradual and less severe, usually presenting after the age of 40 years.

Clinical symptoms

The typical signs and symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus, weakness, and fatigue. These features are more pronounced in type 1 than in type 2 DM, and are a result of hyperglycemia. The complications of DM include retinopathy, nephropathy, neuropathy, macrovascular disease, and impaired wound healing (Lalla *et al*. 2000; Soory 2000a). The treatment of DM is aimed at reducing blood glucose levels to prevent such complications.

There is conclusive evidence of the importance of glycemic control in the prevention of diabetic complications. Patients regularly use blood glucose monitors to provide effective feedback for adjustment of insulin dosage to meet individual requirements (Mealey 1998). Recent studies have shown significant improvement in reducing complications associated with type 2 DM with controlled blood glucose levels (UKPDS 1998a,b). In these studies of over 5000 type 2 DM patients, the risk of retinopathy and nephropathy was reduced by 25% with effective glycemic control, using sulfonylureas, metformin or insulin. The risk of developing hypoglycemia needs to be monitored in these patients on intensive treatment regimes, particularly those on insulin.

Oral and periodontal effects

Poorly controlled diabetic subjects may complain of diminished salivary flow and burning mouth or tongue. Diabetic subjects on oral hypoglycemic agents may suffer from xerostomia, which could predispose to opportunistic infections with *Candida albicans*. Candidiasis has been reported in patients with poorly controlled DM (Ueta *et al*. 1993), associated with suppressed oxygen free radical release by polymorphonuclear cells (PMNs) and reduced phagocytosis.

There is good evidence to support the concept that there is an association between poorly controlled diabetes mellitus and periodontitis (Fig. 12-1). Any differences in periodontal health between type 1 and type 2 DM patients may relate to differences in management of glycemic control, age, duration of disease, utilization of dental care, periodontal disease susceptibility, and habits such as smoking. Type 1 DM patients have an increased risk of developing periodontal disease with age, and with the severity and duration of their diabetes.

Periodontal attachment loss has been found to occur more frequently in moderate and poorly controlled diabetic patients, of both type 1 and type 2 DM, than in those under good control (Westfelt *et al*. 1996). In addition, diabetics with more advanced systemic complications present with a greater frequency and severity of periodontal disease (Karjalainen *et al*. 1994). Conversely, initial phase periodontal treatment comprising motivation and debridement of periodontal pockets in type 2 diabetic patients resulted in improved metabolic control of diabetes (Stewart *et al*. 2001). A recent study by Kiran *et al*. (2005) confirmed these findings. In a study population of patients with type 2 DM and glycosylated hemoglobin values of 6–8%, initial phase periodontal treatment resulted in a significant improvement in glycaemic control. Total cholesterol, triglyceride, and low density lipoprotein levels also decreased in the test group and increased in the control group. These

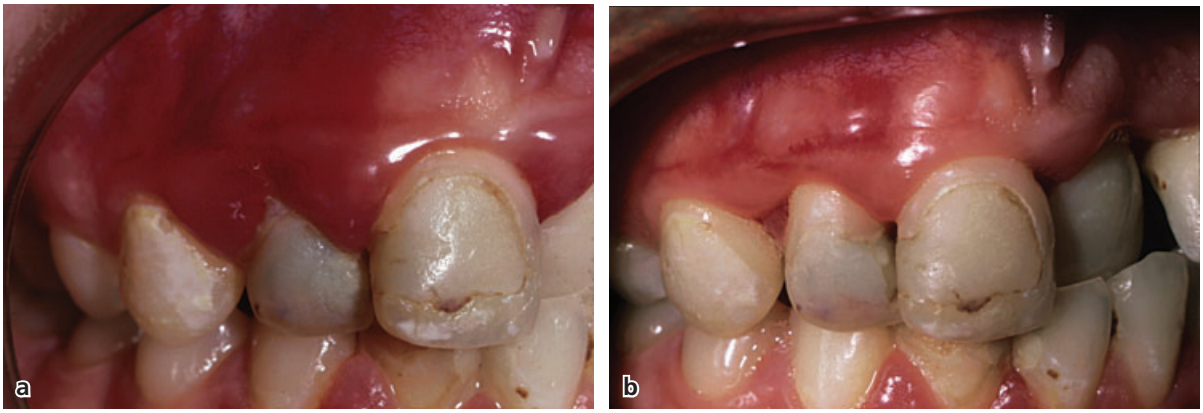

Fig. 12-1 Poorly controlled type 1 diabetes mellitus in a young female aged 19 years. (a) Very inflamed and swollen gingival tissues. Early attachment loss was present. (b) The same patient after responding to a course of non-surgical periodontal treatment and improved oral hygiene.

findings demonstrate that the status of periodontal disease control can contribute to metabolic control of DM (Faria-Almeida *et al*. 2006). The release of cytokines such as tumor necrosis factor (TNF)-α have implications on glucose and lipid metabolism (Cutler & Iacopino 2005) relevant to DM (Iacopino 2001) and cardiovascular disease. There are similar potential interactions between other systemic conditions and oral diseases (Pihlstrom *et al*. 2005; Kinane *et al*. 2006; Meurman & Hamalainen 2006). Insulin resistance can develop in response to chronic bacterial infection seen in periodontal disease, resulting in worse metabolic control in diabetic patients (Grossi *et al*. 1996). There is evidence to support the hypothesis that adequate control of severe inflammatory periodontal disease could alleviate symptoms of co-existing systemic diseases in susceptible individuals. In a population of Pima Indians with type 2 DM and severe periodontal disease, the risk of cardiorenal mortality and diabetic nephropathy was three times greater than amongst those with mild or moderate disease (Saremi *et al*. 2005).

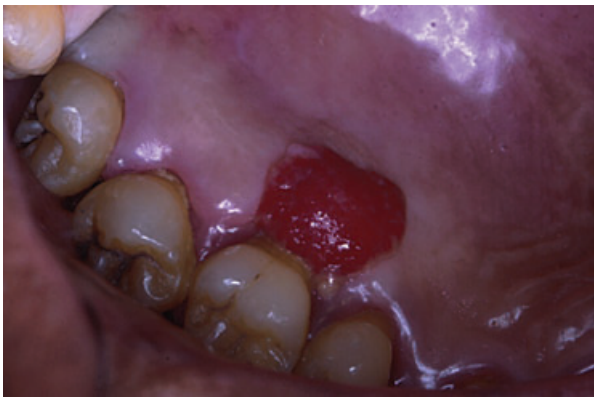

Fig. 12-2 A localized palatal periodontal abscess associated with a periodontal pocket in a 42-year-old poorly controlled diabetic patient.

Probably the most classic description of the undiagnosed or poorly controlled diabetic is the patient presenting with multiple periodontal abscesses, leading to rapid destruction of periodontal support (Figs. 12-2, 12-3). Harrison *et al*. (1983) reported a case of deep neck infection of the submental, sublingual, and submandibular spaces, secondary to periodontal abscesses involving the mandibular incisors, in a poorly controlled diabetic patient. In a population study Ueta *et al*. (1993) demonstrated that DM was a predisposing factor for periodontal and periapical abscess formation due to suppression of neutrophil function. The effects on the host response, and in particular neutrophil function, may account for this finding.

Association of periodontal infection and diabetic control

The presence of acute infection can predispose to insulin resistance (Atkinson & Maclaren 1990). This can occur independently of a diabetic state and persist for up to 3 weeks after resolution of the infection (Yki-Jarvinen *et al*. 1989). In a longitudinal study of subjects with type 2 DM, it was demonstrated that subjects with severe periodontal disease demonstrated significantly worse control of their diabetic condition than those with minimal periodontal

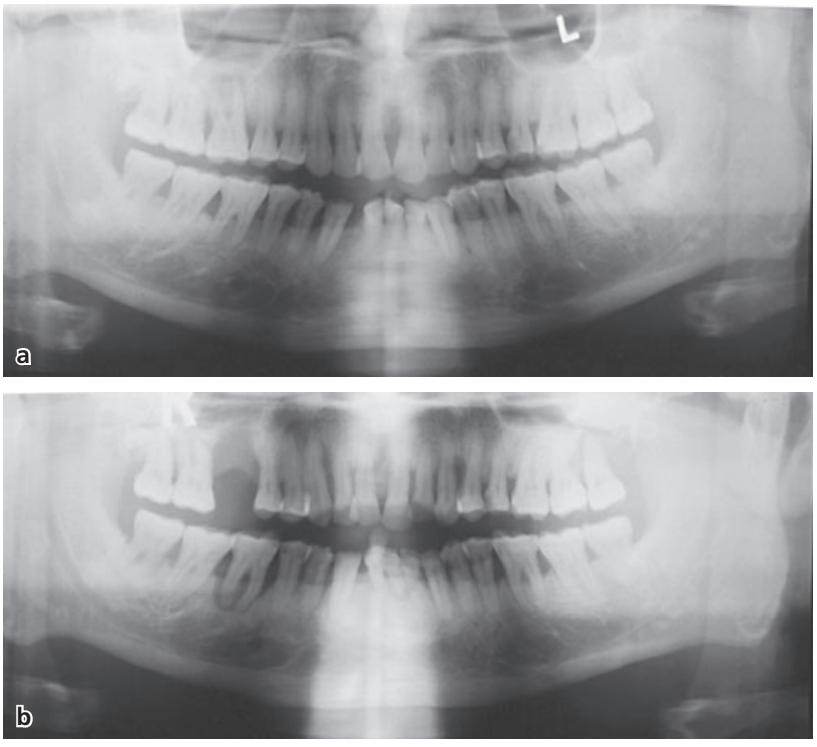

Fig. 12-3 Radiographs of a 50-year-old male who developed type 2 diabetes mellitus in the period between the two radiographs which were taken 3 years apart. There has been rapid bone loss and tooth loss associated with recurrent multiple periodontal abscesses.

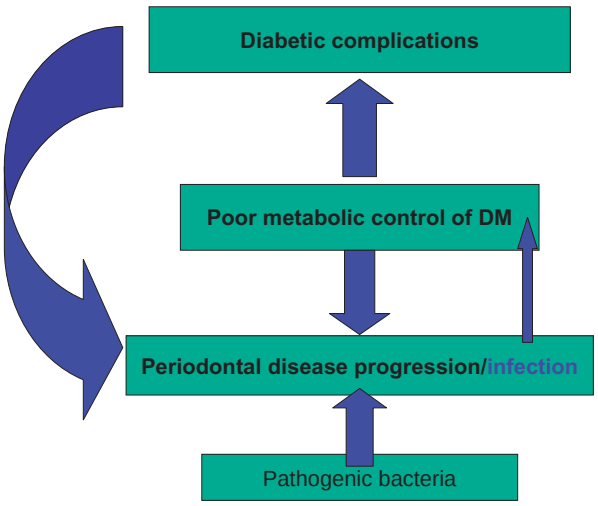

Fig. 12-4 Diabetes control and periodontal disease progression.

involvement (Taylor *et al*. 1996) (Fig. 12-4). The incidence of proteinuria and cardiovascular complications, as a result of uncontrolled diabetes, was found to be significantly greater in diabetics with severe periodontal disease than those with gingivitis or early periodontal disease (Thorstensson *et al*. 1996). Some studies have shown that stabilization of the periodontal condition with mechanical therapy, in combination with systemic tetracycline, improves the diabetic condition in such patients (Grossi *et al*. 1997b). Reduced insulin dosage in type 1 diabetics following periodontal treatment has also been reported (Sastrowijoto *et al*. 1990). However, other studies have not shown improvement in diabetic control following non-surgical periodontal treatment (Aldridge *et al*. 1995). These effects of periodontal therapy may be more pronounced in poorly controlled diabetic patients with severe periodontal disease.

Significant inflammatory lesions in severe periodontal disease could contribute to exacerbation of diabetes. Markers of inflammation common to diabetes and periodontal disease are an indication of disease control (Soory 2002, 2004).

Modification of the host–bacteria relationship in diabetes

Effects on microbiota

Hyperglycemia in uncontrolled diabetics has implications on the host response (Gugliucci 2000) and affects the regional microbiota. This can potentially influence the development of periodontal disease and caries in poorly controlled type 1 and type 2 DM patients. *Capnocytophaga* species have been isolated as the predominant cultivable organisms from periodontal lesions in type 1 diabetics, averaging 24% of the cultivable flora (Mashimo *et al*. 1983). A similar distribution of the predominant putative pathogens, *Prevotella intermedia*, *Campylobacter rectus*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* (formerly known as *Actinobacillus actinomycetemcomitans*), to those associated with chronic adult periodontal disease was detected in periodontal lesions of type 2 diabetics (Zambon *et al*. 1988), with potential for disease activity during poor metabolic control. In an insulin-dependent diabetic population with a large proportion of poorly controlled diabetics, Seppala and Ainamo (1996) showed significantly increased percentages of spirochetes and motile rods and decreased levels of cocci in periodontal lesions, compared with well controlled patients.

Effects on the host response

Diabetes mellitus has far-reaching effects on the host response (Fig. 12-5).

Polymorphonuclear leukocytes

Reduced PMN function (Marhoffer *et al*. 1992) and defective chemotaxis in uncontrolled diabetics can contribute to impaired host defenses and progression of infection (Ueta *et al*. 1993). Crevicular fluid collagenase activity, originating from PMNs, was found to be increased in diabetic patients and this could be inhibited *in vitro* by tetracycline through its enzyme inhibitory effects (Sorsa *et al*. 1992). The PMN enzymes beta-glucuronidase (Oliver *et al*. 1993) and elastase, in association with diabetic angiopathy (Piwowar *et al*. 2000), have been detected at significantly higher levels in poorly controlled diabetic patients.

Cytokines, monocytes, and macrophages

Diabetic patients with periodontitis have significantly higher levels of interleukin (IL)-1β and prostaglandin E_2 (PGE_2) in crevicular fluid compared to non-diabetic controls with a similar degree of periodontal disease (Salvi *et al*. 1997). In addition, the release of these cytokines (IL-1β, PGE_2, TNF-α) by monocytes has been shown to be significantly greater in diabetics than in non-diabetic controls. Chronic hyperglycemia results in non-enzymatic glycosylation of numerous proteins, leading to the accumulation of advanced glycation end products (AGE), which play a central role in diabetic complications (Brownlee 1994). Increased binding of AGEs to macrophages and monocytes (Brownlee 1994) can result in a destructive cell phenotype with increased sensitivity to stimuli, resulting in excessive release of cytokines. Altered macrophage phenotype due to cell surface binding with AGE, prevents the development of macrophages associated with repair. This could contribute to delayed wound healing seen in diabetic patients (Iacopino 1995).

Connective tissue

A hyperglycemic environment, due to decreased production or utilization of insulin, can reduce growth,

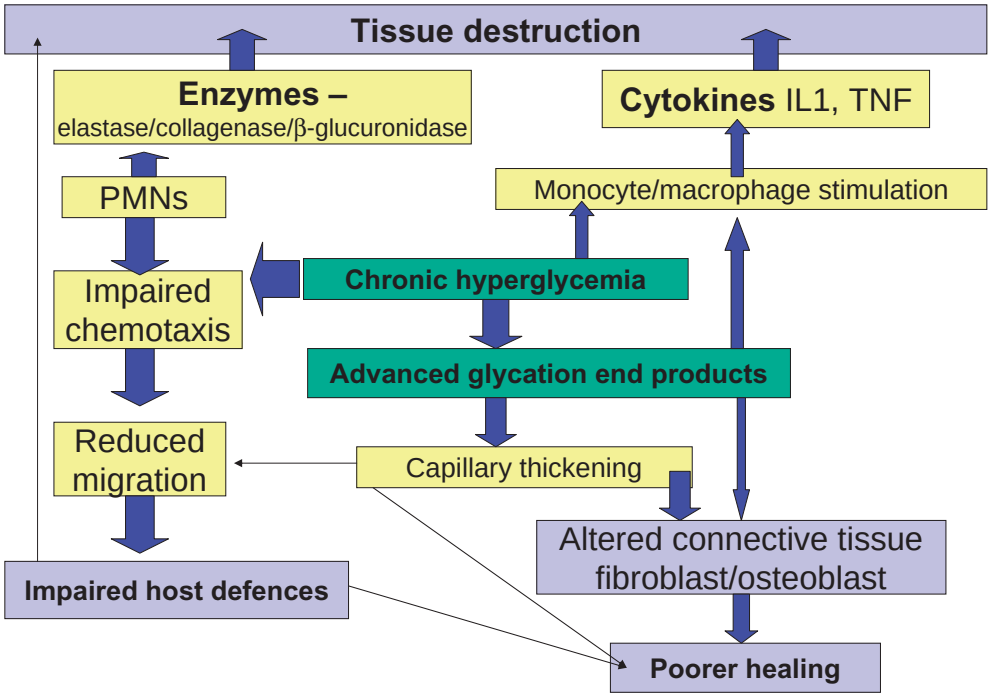

Fig. 12-5 Effects of diabetes mellitus on the host response.

proliferation, and matrix synthesis by gingival and periodontal ligament fibroblasts and osteoblasts. The formation of AGE results in reactive oxygen species, which are damaging to cellular function in gingival tissues, due to oxidative stress (Schmidt *et al*. 1996). The accumulation of AGE in tissues alters the function of several intercellular matrix components, including vascular wall collagen, resulting in deleterious complications (Ulrich & Cerami 2001). This has adverse effects on cell–matrix interactions and vascular integrity, potentially affecting periodontal disease presentation and treatment responses in uncontrolled diabetics. Vascular changes, such as thickening of the capillary basement membrane in a hyperglycemic environment, can impair oxygen diffusion, metabolic waste elimination, PMN migration, and diffusion of antibodies. Binding of AGE to vascular endothelial cells can trigger responses that induce coagulation, leading to vasoconstriction and microthrombus formation (Esposito *et al*. 1992), resulting in impaired perfusion of tissues. Recent work using a cell culture model has demonstrated that glucose, AGE, and nicotine inhibit the synthesis of steroid markers of wound healing (Rahman & Soory 2006). This inhibition was overcome by the antioxidant glutathione and insulin-like growth factor (IGF), which also functions as an antioxidant. These findings can be extrapolated to the 'in vivo' situation, demonstrating the relevance of oxidative stress-induced mechanisms in periodontal disease and DM, with therapeutic implications of medications with antioxidant effects (Soory & Tilakaratne 2003). These findings may be extrapolated to healing responses in the uncontrolled diabetic smoker with periodontal disease (Graves *et al*. 2006).

Effects on healing and treatment response

Wound healing is impaired due to the cumulative effects on cellular functions as described above. In summary, these factors include:

1. Decreased synthesis of collagen by fibroblasts
2. Increased degradation by collagenase
3. Glycosylation of existing collagen at wound margins
4. Defective remodeling and rapid degradation of newly synthesized, poorly cross-linked collagen.

Periodontal treatment

The treatment of well controlled DM patients would be similar to that of non-diabetic patients for most routine dental procedures. The short-term non-surgical treatment response of stable diabetics has been found to be similar to that of non-diabetic controls, with similar trends in improved probing depths, attachment gain, and altered subgingival microbiota (Christgau *et al*. 1998). Well controlled diabetics with regular supportive therapy have been shown to maintain treatment results 5 years after a combination of non-surgical and surgical treatment (Westfelt *et al*. 1996). However, a less favorable treatment outcome may occur in long-term maintenance therapy of poorly controlled diabetics, who may

succumb to more rapid recurrence of initially deep pockets (Tervonen & Karjalainen 1997).

Puberty, pregnancy, and the menopause

The hormonal variations experienced by women during physiological and non-physiological conditions (such as hormone replacement therapy and use of hormonal contraceptives) result in significant changes in the periodontium, particularly in the presence of pre-existing, plaque-induced gingival inflammation. The implications of these changes on the tissues of the periodontium have been reviewed comprehensively (Mascarenhas *et al*. 2003; Guncu *et al*. 2005). Periods of hormonal flux are known to occur during puberty, menstruation, pregnancy, and the menopause. Changes in hormone levels occur when the anterior pituitary secretes follicle-stimulating hormone (FSH) and luteinizing hormone (LH), resulting in the maturation of the ovary and cyclical production of estrogen and progesterone.

The gingiva is a target tissue for the actions of steroid hormones. Clinical changes in the tissues of the periodontium have been identified during periods of hormonal fluctuation. The effects of estrogen and progesterone on the periodontium have received significant research attention. The main potential effects of these hormones on the periodontal tissues can be summarized as:

- Estrogen affects salivary peroxidases, which are active against a variety of microorganisms (Kimura *et al*. 1983), by changing the redox potential.
- Estrogen has stimulatory effects on the metabolism of collagen and angiogenesis (Sultan *et al*. 1986).
- Estrogen can trigger autocrine or paracrine polypeptide growth factor signaling pathways, whose effects may be partially mediated by the estrogen receptor itself (Chau *et al*. 1998).
- Estrogen and progesterone can modulate vascular responses and connective tissue turnover in the periodontium, associated with interaction with inflammatory mediators (Soory 2000b).

The interaction of estrogen and progesterone with inflammatory mediators may help to explain the increased levels of inflammation seen during periods of hormonal fluctuation. For example, when cultured human gingival fibroblasts were incubated with progesterone concentrations common in late pregnancy, there was a 50% reduction in the formation of the inflammatory mediator IL-6, compared with control values (Lapp *et al*. 1995). IL-6 induces the synthesis of tissue inhibitor of metalloproteinases (TIMP) in fibroblasts (Lotz & Guerne 1991), reduces the levels of TNF and enhances the formation of acute phase proteins (Le & Vilcek 1989). A progesterone-induced reduction in IL-6 levels could result in less TIMP, more proteolytic enzyme activity, and higher levels of TNF at the affected sites, due to less inhibition, resulting in inflammation and obvious clinical manifestations.

Puberty and menstruation

During puberty, there are raised levels of testosterone in males and estradiol in females. Several studies have demonstrated an increase in gingival inflammation in children of circumpubertal age, with no change in plaque levels (Sutcliffe 1972). In a longitudinal study, Mombelli *et al*. (1989) reported that the mean papillary bleeding scores and percentage of interdental bleeding sites correlated with the development of secondary sexual characteristics at puberty, while other studies did not find a significant correlation between the onset of puberty and gingival changes in parapubescent women (Tiainen *et al*. 1992). These discrepancies may be attributed to factors such as the oral hygiene status of the population and study design.

The prevalence of certain periodontal pathogens reported during puberty may have a direct association with the hormones present and their utilization by selected pathogens. For example *Prevotella intermedia* is able to substitute progesterone and estrogen for menadione (vitamin K) as an essential nutrient (Kornman & Loesche 1979). An association between pubertal gingivitis, *P. intermedia* and serum levels of testosterone, estrogen, and progesterone has been reported in a longitudinal study (Nakagawa *et al*. 1994).

Pre-existing plaque-induced gingivitis may be an important factor in detecting hormone-induced changes during the menstrual cycle. Holm-Pedersen and Loe (1967) demonstrated that women with gingivitis experienced increased inflammation with an associated increase in crevicular fluid exudate during menstruation compared with healthy controls. Most female patients are not aware of any changes in their gingivae during the menstrual cycle (Amar & Chung 1994), while a few experience enlarged hemorrhagic gingivae in the days preceding menstrual flow. This has been associated with more gingivitis, increased crevicular fluid flow, and tooth mobility (Grant *et al*. 1988). Early studies demonstrated similar findings during the menstrual cycle in a population with pre-existing gingivitis, in response to fluctuations in the levels of estrogen and progesterone (Lindhe & Attstrom 1967).

Pregnancy

During pregnancy, the increased levels of sex steroid hormones are maintained from the luteal phase which results in implantation of the embryo, until parturition. Pregnant women, near or at term, produce large quantities of estradiol (20 mg/day), estriol (80 mg/day), and progesterone (300 mg/day).

Gingival inflammation initiated by plaque, and exacerbated by these hormonal changes in the second and third trimester of pregnancy, is referred to as pregnancy gingivitis. Parameters, such as gingival probing depths (Hugoson 1970; Miyazaki *et al*. 1991), bleeding on probing (Miyazaki *et al*. 1991), and crevicular fluid flow (Hugoson 1970), were found to be increased. These inflammatory features can be minimized by maintaining good plaque control.

According to early reports, the prevalence of pregnancy gingivitis ranges from 35% (Hasson 1966) to 100% (Lundgren *et al*. 1973). In a study of 130 pregnant women, Machuca *et al*. (1999) demonstrated gingivitis in 68% of the population, ranging from 46% in technical executives to 88% in manual workers. Cross-sectional studies examining pregnant and postpartum women have shown that pregnancy is associated with significantly more gingivitis than at postpartum, despite similar plaque scores (Silness & Loe 1963). Further observations were made by Hugoson (1970) in a longitudinal study of 26 women during and following pregnancy, which also demonstrated that the severity of gingival inflammation correlated with the gestational hormone levels during pregnancy (Fig. 12-6). A more recent study of a rural population of Sri Lankan women (Tilakaratne *et al*. 2000a) showed increased gingivitis of varying degrees of significance amongst all the pregnant women investigated, compared with matched non-pregnant controls. There was a progressive increase in inflammation with advancing pregnancy which was more significant in the second and third trimesters of pregnancy, despite the plaque levels remaining unchanged. At the third month after parturition, the level of gingival inflammation was similar to that observed in the first trimester of pregnancy. This suggests a direct correlation between gingivitis and sustained, raised levels of gestational hormones during pregnancy, with regression during the postpartum period. In investigations by Cohen *et al*. (1969) and Tilakaratne *et al*. (2000a), the values for loss of attachment remained unchanged during pregnancy and 3 months postpartum.

Effects on the microbiota

There is an increase in the selective growth of periodontal pathogens such as *P. intermedia* in subgingival plaque during the onset of pregnancy gingivitis at the third to fourth month of pregnancy. The gestational hormones act as growth factors, by satisfying the naphthoquinone requirement for bacteria (Di Placido *et al*. 1998). These findings were also confirmed by Muramatsu and Takaesu (1994) who showed that from the third to fifth month of pregnancy, the number of gingival sites which bled on probing corresponded with the percentage increase in *P. intermedia*. During pregnancy, progesterone is less actively catabolized to its inactive products, resulting in higher levels of the active hormone (Ojanotko-Harri *et al*. 1991). A 55-fold increase in the proportion of *P. intermedia* has been demonstrated in pregnant women compared with non-pregnant controls (Jensen *et al*. 1981), implying a role for gestational hormones in causing a change in microbial ecology in the gingival pocket. Although an overall association has been demonstrated, a cause and effect relationship may be less clear.

Effects on the tissues and host response

The increase in severity of gingivitis during pregnancy has been partly attributed to the increased circulatory levels of progesterone and its effects on the capillary vessels (Lundgren *et al*. 1973). Elevated progesterone levels in pregnancy enhance capillary permeability and dilatation, resulting in increased gingival exudate. The effects of progesterone in stimulating prostaglandin synthesis can account for some of the vascular changes (Miyagi *et al*. 1993).

The elevated levels of estrogen and progesterone in pregnancy affect the degree of keratinization of the

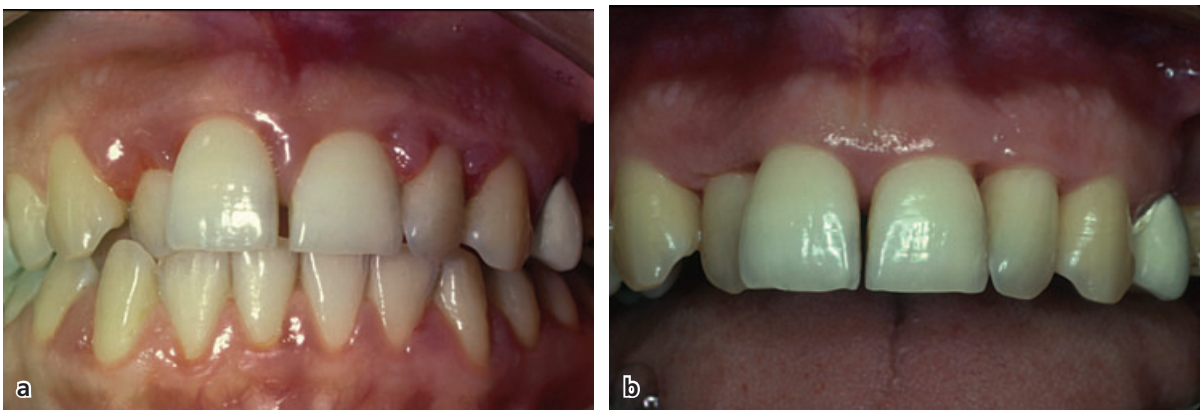

Fig. 12-6 Gingivitis associated with pregnancy. (a) A patient in the last trimester of pregnancy with very inflamed edematous gingival tissue which tended to bleed with the slightest provocation. (b) The improvement in gingival health 6 months after birth of the baby and an intensive course of non-surgical periodontal treatment.

gingival epithelium and alter the connective tissue ground substance. The decreased keratinization of the gingivae, together with an increase in epithelial glycogen, are thought to result in decreased effectiveness of the epithelial barrier in pregnant women (Abraham-Inpijn *et al*. 1996). Hormonal factors that affect the epithelium and increase vascular permeability can contribute to an exaggerated response to bacterial plaque during pregnancy. The influence of gestational hormones on the immune system can contribute further to the initiation and progression of pregnancy gingivitis. High levels of progesterone and estrogen associated with pregnancy (and the use of some oral contraceptives) have been shown to suppress the immune response to plaque (Sooriyamoorthy & Gower 1989). Neutrophil chemotaxis and phagocytosis, along with antibody and T cell responses, have been reported to be depressed in response to high levels of gestational hormones (Raber-Durlacher *et al*. 1993).

Pregnancy granuloma or epulis

A pedunculated, fibrogranulomatous lesion can sometimes develop during pregnancy and is referred to as a pregnancy granuloma or epulis. A combination of the vascular response induced by progesterone and the matrix stimulatory effects of estradiol contributes to the development of pregnancy granulomas, usually at sites with pre-existing gingivitis (Fig. 12-7). The vascular effects result in a bright red, hyperemic, and edematous presentation. The lesions often occur in the anterior papillae of the maxillary teeth and usually do not exceed 2 cm in diameter. They can bleed when traumatized and their removal is best deferred until after parturition, when there is often considerable regression in their size (Wang *et al*. 1997). Surgical removal of the granuloma during pregnancy can result in recurrence due to a combination of poor plaque control and hormone-mediated growth of the lesion. Careful oral hygiene and debridement during pregnancy are important in preventing its occurrence (Wang *et al*. 1997).

Periodontal treatment during pregnancy

Pregnant women need to be educated on the consequences of pregnancy on gingival tissues and thoroughly motivated in plaque control measures, with professional treatment as required. They are likely to be more comfortable to receive dental treatment during the second trimester than in the first or third trimester of pregnancy, although emergency treatment is permissible at any stage during pregnancy (Amar & Chung 1994). Since most medications cross the placental barrier and organogenesis occurs mainly in the first trimester, pregnant women are best treated in the second trimester, to avoid the occurrence of developmental defects. Any form of medication during pregnancy must only be used if the gravity of the condition being treated outweighs the consequences. Amongst the antibiotics, tetracycline, vancomycin, and streptomycin can contribute to staining of teeth and ototoxic and nephrotoxic effects during the fourth to ninth months of pregnancy; erythromycin, penicillins, and cephalosporins are relatively safer, but any medication must only be administered in consultation with the patient's obstetrician (Lynch *et al*. 1991).

Menopause and osteoporosis

During menopause there is a decline in hormonal levels due to decreased ovarian function. This is characterized by tissue changes such as desquamation of gingival epithelium (Fig. 12-8) and osteoporosis (Fig. 12-9) which may be attributed to hormone deficiency. It has been demonstrated that women with early onset of menopause have a higher incidence of osteoporosis and significantly lower bone mineral density (Kritz-Silverstein & Barrett-Connor 1993).

A third of women over age 60 are affected by postmenopausal osteoporosis (Baxter 1987). The changes involved are a reduction in bone density, affecting its mass and strength without significantly affecting its chemical composition. An alteration in the calcium–phosphate equilibrium due to deficient

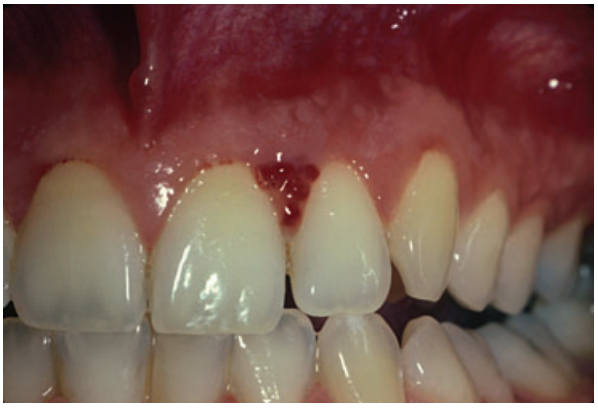

Fig. 12-7 Multi-lobulated appearance of an early pregnancy epulis, demonstrating vascular elements and tissue oedema.

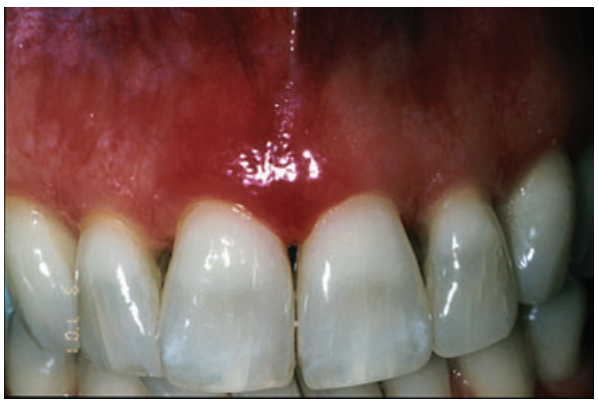

Fig. 12-8 Clinical appearance of anterior maxillary gingiva with pronounced desquamation in a woman during menopause.

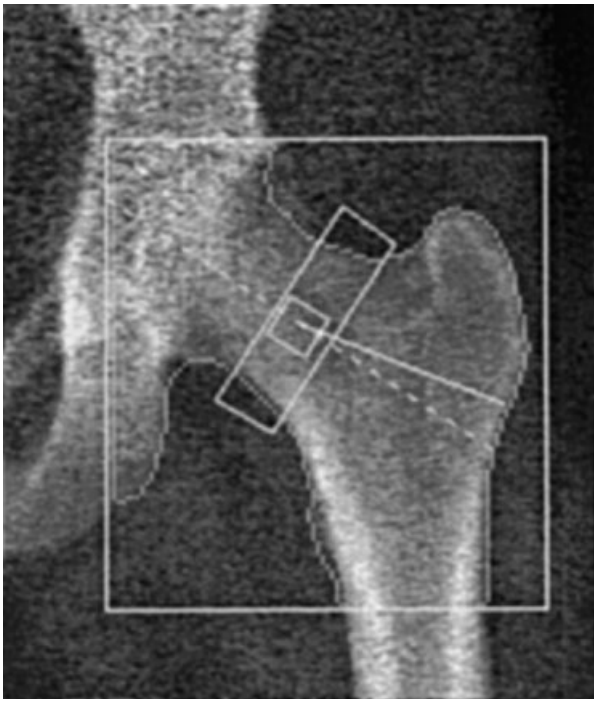

Fig. 12-9 A DEXA scan used to measure mineral bone density in the hip. This technique is not routinely applied to the jaws.

absorption of dietary calcium and increased excretion due to diminished estrogen levels can account for some of the bone changes seen in postmenopausal women (Shapiro *et al*. 1985), usually involving the mandible more than the maxilla.

Estrogen replacement therapy has been shown to prevent osteoporosis and maintain bone mineral content at several sites throughout the skeleton (Moore *et al*. 1990), with a 5% increase in bone mineral content in the region of the head compared to those taking placebo (Gotfredsen *et al*. 1986). The influence of estrogen on bone mineral density has been demonstrated in these studies, but a cause and effect relationship with periodontal disease is less clear.

A 2-year follow-up study of 42 171 postmenopausal women (Grodstein *et al*. 1996) showed that the risk of tooth loss was significantly lower amongst hormone users. These findings reinforce those of Paganini-Hill (1995), who showed a 36% decrease in tooth loss in estrogen users compared with non-users. There is evidence to suggest that use of estrogen is necessary to protect against bone loss (Grady *et al*. 1992). Although osteoporosis in postmenopausal women may not be the cause of periodontal disease, it may affect the severity of pre-existing disease. The circulating levels of estrogen have been shown to have an influence on alveolar bone density in postmenopausal women (Payne *et al*. 1997).

Effect of smoking on osteoporosis

A negative association between smoking and bone density has been demonstrated by Krall and Dawson-Hughes (1991). Smokers can differ from non-smokers in weight, caffeine intake, age at menopause, and alcohol consumption (Lindquist & Bengtsson 1979; Rigotti 1989); all these factors can potentially confound an association between smoking and bone density. A study on female twins by Hopper and Seeman (1994) showed that in the 20 pairs who varied most, by 20 or more pack years, the differences in bone density within pairs were 9.3% at the lumbar spine, 5.8% at the femoral neck, and 6.5% at the femoral shaft. This study also demonstrated increased serum levels of FSH and LH in smokers, implying reduced circulating levels of estrogen, leading to increased bone resorption. Other investigators have demonstrated the effects of smoking on the synthesis and degradation of estrogen (Jensen *et al*. 1985). The study by Jensen *et al*. (1985) investigated 136 postmenopausal women who were treated with three different doses of estrogen–progesterone or placebo. They showed reduced levels of estrogen in smokers (range of 1–30 cigarettes/day in the previous 6 months, mean 12.4), compared with non-smokers (not smoked in the previous 3 months). There was also a significant inverse correlation between the number of cigarettes smoked per day and the serum levels of estrogen, suggestive of increased hepatic metabolism of estrogen in postmenopausal smokers, resulting in lower serum levels of these hormones.

Treatment of osteoporosis

In osteoporotic patients, the rate of bone loss during the early postmenopausal period increases to 3–4% per year. Estrogen replacement therapy, which slows bone turnover, results in increased bone density in the trabecular spaces during remodeling (Frost 1989). The increased skeletal bone mass which occurs in response to estrogen replacement therapy is apparent in the first 2 years of treatment and maintained with continuation of treatment (Kimmel *et al*. 1994). The effects of estrogen in regaining bone mass to premenopausal levels and in preventing/reversing postmenopausal osteoporotic changes in the long bones and spine have been demonstrated in several studies (Armamento-Villareal *et al*. 1992; Takahashi *et al*. 1994).

There is some controversy with regard to the benefits of hormone replacement due to the risk factors involved. Fractures due to osteoporosis and heart disease in postmenopausal women can be reduced by 50% with estrogen replacement therapy. However, hormone replacement with estrogen alone exposes such patients to the risk of endometrial cancer. Long-term hormone replacement therapy has been shown to correlate with an increased risk of breast cancer. Modern formulations utilize combined therapy with a suitable dose of progesterone in combination with estrogen in order to minimize some of these risk factors (Whitehead & Lobo 1988).

Hormonal contraceptives

Contraceptives utilize synthetic gestational hormones (estrogen and progesterone), to reduce the likelihood of ovulation/implantation (Guyton 1987). Less dramatic but similar effects to pregnancy are sometimes observed in the gingivae of hormonal contraceptive users. The most common oral manifestation of elevated levels of ovarian hormones is an increase in gingival inflammation with an accompanying increase in gingival exudate (Mariotti 1994).

There are reported systemic risk factors associated with long-term use of hormonal contraceptives. The correlation between hormonal contraceptive use and significant cardiovascular disease associated with arterial and venous thromboembolic episodes has been reviewed by Westhoff (1996). Estrogen is responsible for both arterial and venous effects, while progesterone effects arterial changes. Women using oral contraceptives show elevated plasma levels of several clotting factors, related to the dose of estrogen. Raised levels of factors VIIc and XIIc are significant, since they increase the likelihood of coagulation and in men these factors have a strong positive correlation with ischemic heart disease. However, the relative risk is dependent on the contraceptive formulation used and there may not be a consistent biological plausibility to explain this association (Davis 2000).

There are several different formulations of hormonal contraceptives (Davis 2000) including:

1. Combined oral contraceptives containing artificial analogues of estrogen and progesterone
2. Progesterone-based mini-pill
3. Slow release progesterone implants placed subdermally that last up to 5 years (e.g. Norplant)
4. Depo Provera, a very effective progestin injection given by a doctor every 3 months.

Current combined oral contraceptives consist of low doses of estrogens (50 µg/day) and/or progestins (1.5 mg/day) (Mariotti 1994). The formulations used in the early periodontal studies contained higher concentrations of gestational hormones, e.g. 50 µg estrogen with 4 mg progestin (El-Ashiry *et al*. 1971), 100 µg estrogen with 5 mg progestin (Lindhe & Bjorn 1967). The results obtained in these studies would partly reflect the contraceptive preparation used. In one early study (Knight & Wade 1974) women who were on hormonal contraceptives for more than 1.5 years exhibited greater periodontal destruction compared to the control group of comparable age and oral hygiene. This could partly reflect higher dose of gestagens used in older contraceptive preparations. However, a recent study on a population of rural Sri Lankan women confirmed these findings (Tilakaratne *et al*. 2000b), showing significantly higher levels of gingivitis in contraceptive users (0.03 mg estradiol and 0.15 mg of a progestin), than non-users, despite similar plaque scores. There was also significant periodontal breakdown in those who used the progesterone injection (a depot preparation of 150 mg progesterone) 3-monthly for 2–4 years, compared with those who used it for less than 2 years. These findings may be attributed to the duration of use, and the effects of progesterone in promoting tissue catabolism, resulting in increased periodontal attachment loss. However, if low plaque levels are established and maintained for the duration of use, these effects could be minimized.

Effect on tissue response

Both estrogen and progesterone are known to cause increased gingival exudate, associated with inflammatory edema (Lindhe & Bjorn 1967). A 53% increase in crevicular fluid volume has been demonstrated in hormonal contraceptive users compared with controls. El-Ashiry *et al*. (1971) observed that the most pronounced effects on the gingiva occurred in the first 3 months of contraceptive treatment, but the dose of gestational hormones was higher in the older formulations compared with those used currently (Davis 2000), accounting for a more florid response in the tissues.

It has been suggested that the interaction of estrogen with progesterone results in the mediation of the effects characteristic of progesterone. Human gingiva has receptors for progesterone and estrogen (Vittek *et al*. 1982; Staffolani *et al*. 1989), providing evidence that gingiva is a target tissue for both gestational hormones. In *in vitro* studies of cultured gingival fibroblasts, estrogen enhanced the formation of anabolic androgen metabolites, while progesterone caused a diminished response. The combined effect of both gestational hormones on the yield of androgens was less pronounced than with estrogen alone, implying a more catabolic role for progesterone (Tilakaratne & Soory 1999).

Progesterone causes increased vascular permeability, resulting in the infiltration of polymorphonuclear leukocytes and raised levels of PGE_2 in the sulcular fluid (Miyagi *et al*. 1993). Increased capillary permeability may be induced by estrogen by stimulating the release of mediators such as bradykinin, prostaglandins, and histamine. However, the main effects of estrogen are in controlling blood flow. Hence the combination of estrogen and progesterone in the contraceptive pill can contribute to vascular changes in the gingivae. The resultant gingivitis can be minimized by establishing low plaque levels at the beginning of oral contraceptive therapy (Zachariasen 1993).

Tobacco smoking

Tobacco smoking is very common, with cigarettes being the main product smoked. In the European Union, an average of 29% of the adult population smoke, ranging from 17.5% in Sweden to 45% in Greece (http://www.ash.org.uk). The figure is higher

for men (35%) than for women (24%). Most smokers start the habit as teenagers, with the highest prevalence in the 20–24-year-old age group. Socioeconomic differences also exist with higher smoking in the lower socioeconomic groups. These data are similar for the US population (Garfinkel 1997; http://www.cdc.gov/tobacco/) where an estimated 44.5 million adults smoke. Reported smoking rates for third world countries are even higher. Smoking is associated with a wide spectrum of disease including stroke, coronary artery disease, peripheral artery disease, gastric ulcer, and cancers of the mouth, larynx, esophagus, pancreas, bladder, and uterine cervix. It is also a major cause of chronic obstructive pulmonary disease and a risk factor for low birth weight babies. Approximately 50% of regular smokers are killed by their habit and smoking causes 30% of cancer deaths.

Cigarette smoke is a very complex mixture of substances with over 4000 known constituents. These include carbon monoxide, hydrogen cyanide, reactive oxidizing radicals, a high number of carcinogens, and the main psychoactive and addictive molecule – nicotine (Benowitz 1996). Many of these components could modify the host response in periodontitis. In most of the *in vitro* studies considered in the latter parts of this chapter the experimenters utilized simple models with nicotine alone. Tobacco smoke has a gaseous phase and solid phase which contains tar droplets. The tar and nicotine yields of cigarettes have been reduced due to physical characteristics of the filters. However, there has been little change in the tar and nicotine content of the actual tobacco and the dose an individual receives is largely dependent upon the way in which they smoke (Benowitz 1989). Inter-subject smoking variation includes: frequency of inhalation; depth of inhalation; length of the cigarette stub left; presence or absence of a filter; and the brand of cigarette (Benowitz 1988).

The patient's exposure to tobacco smoke can be measured in a number of ways, including interviewing the subject using simple questions or more sophisticated questionnaires and biochemical analyses (Scott *et al*. 2001). The latter tests include exhaled carbon monoxide in the breath, which is commonly measured in smoking cessation clinics, and cotinine (a metabolite of nicotine) in saliva, plasma/serum or urine (Wall *et al*. 1988). Cotinine measurements are more reliable in determining a subject's exposure to tobacco smoke because the half-life is 14–20 hours compared with the shorter half-life of nicotine which is 2–3 hours (Jarvis *et al*. 1988). The mean plasma and salivary cotinine concentrations of regular smokers are approximately 300 ng/ml and urine concentrations are about 1500 ng/ml. Non-smokers typically have plasma/saliva concentrations under 2 ng/ml, but this may be raised slightly due to environmental exposure (passive smoking).

Inhalation of tobacco smoke allows very rapid absorption of nicotine into the blood and transport to the brain, which is faster than an intravenous infusion. Nicotine in tobacco smoke from most cigarettes is not well absorbed through the oral mucosa because the nicotine is in an ionized form as a result of the pH (5.5). In contrast cigar and pipe smoke is more alkaline (pH 8.5), which allows good absorption of un-ionized nicotine through the buccal mucosa (Benowitz 1988). Nicotine is absorbed rapidly in the lung where the smoke is well buffered. The administration of nicotine causes a rise in the blood pressure, an increase in heart rate, an increase in respiratory rate, and decreased skin temperature due to peripheral vasoconstriction. However, at other body sites, such as skeletal muscle, nicotine produces vasodilatation.

These differing actions of nicotine have led to some controversy over its action in the periodontal tissues. Clarke and co-workers (1981) showed that the infusion of nicotine resulted in a transient decrease in gingival blood flow in a rabbit model. However, Baab and Öberg (1987) using laser Doppler flowmetry to monitor relative gingival flow in 12 young smokers, observed an immediate but transient increase in relative gingival blood flow during smoking, compared to the presmoking or resting measurements. The authors hypothesized that the steep rise in heart rate and blood pressure due to smoking could lead to an increase in the gingival circulation during smoking. These results were confirmed by Meekin *et al*. (2000) who showed that subjects who smoked only very occasionally experienced an increase in blood flow to the head, whereas regular smokers showed no change in blood flow, demonstrating tolerance in the regular smoker. The increase in blood flow to the gingival and forehead skin following an episode of smoking in 13 casual consumers of tobacco was confirmed by Mavropoulos *et al*. (2003) and Morozumi *et al*. (2004) showed that the gingival blood flow significantly increased at 3 days following quitting, providing important information on the recovery of gingival tissue following quitting smoking.

Periodontal disease in smokers

Pindborg (1947) was one of the first investigators to study the relationship between smoking and periodontal disease. He discovered a higher prevalence of acute necrotizing ulcerative gingivitis, a finding that was confirmed in many subsequent studies of this condition (Fig. 12-10) (Pindborg 1949; Kowolik & Nisbet 1983; Johnson & Engel 1986). Early studies showed that smokers had higher levels of periodontitis but they also had poorer levels of oral hygiene (Brandzaeg & Jamison 1984) and higher levels of calculus (Fig. 12-11) (Alexander 1970; Sheiham 1971). Later studies which took account of oral hygiene status and employed more sophisticated statistical analyses showed that smokers had more disease regardless of oral hygiene (Ismail *et al*. 1983; Bergstrom 1989; Bergstrom & Preber 1994).

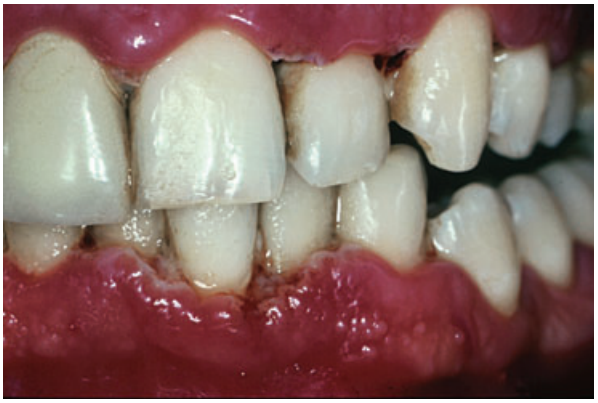

Fig. 12-10 The typical appearance of necrotizing ulcerative gingivitis in a heavy smoker with poor oral hygiene.

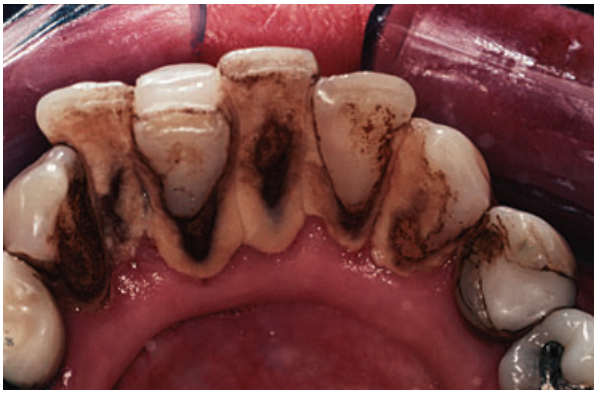

Fig. 12-11 The lingual aspects of the lower incisors showing gross supragingival calculus formation and relatively little gingival inflammation in a female patient who has smoked 20 cigarettes per day for over 20 years.

A large number of studies have established that in comparing smokers and non-smokers with periodontitis, smokers have:

1. Deeper probing depths and a larger number of deep pockets (Feldman *et al*. 1983; Bergstrom & Eliassson 1987a; Bergstrom *et al*. 2000a)
2. More attachment loss including more gingival recession (Grossi *et al*. 1994; Linden & Mullally 1994; Haffajee & Socransky 2001a)
3. More alveolar bone loss (Bergstrom & Floderus Myhred 1983; Bergstrom & Eliasson 1987b; Feldman *et al*. 1987; Bergstrom *et al*. 1991, 2000b; Grossi *et al*. 1995)
4. More tooth loss (Osterberg & Mellstrom 1986; Krall *et al*. 1997)
5. Less gingivitis and less bleeding on probing (Feldman *et al*. 1983; Preber & Bergstrom 1985; Bergstrom & Preber 1986; Haffajee & Socransky 2001a)
6. More teeth with furcation involvement (Mullally & Linden 1996).

The finding of less gingival bleeding on probing is associated with less inflamed marginal tissue and lower bleeding scores when probing the depth of the pockets. The typical clinical appearance of the smoker 's gingival tissue is shown in Fig. 12-12, which demonstrates relatively low levels of marginal inflammation and a tendency to a more fibrotic appearance with little edema. Despite the clinical appearance of the gingival tissue, the patient has deep pockets,

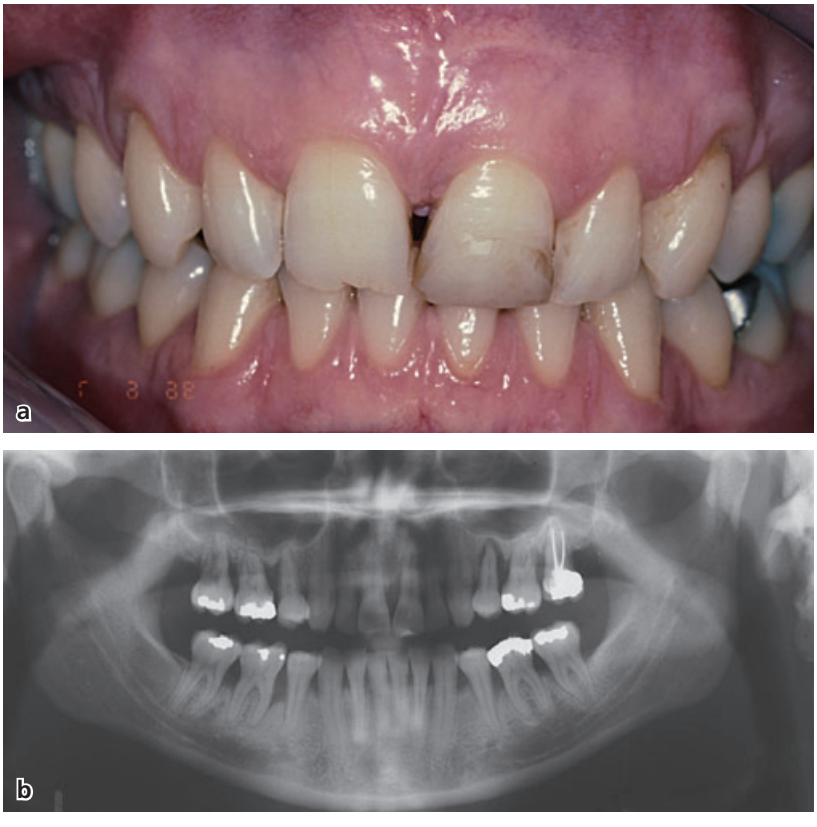

Fig. 12-12 A 30-year-old female smoker with advanced periodontitis. (a) The clinical appearance shows marginal gingiva with little signs of inflammation. Probing depths greater than 6 mm were present at most interproximal sites, but with little bleeding on probing. (b) Generalized advanced bone loss in this patient.

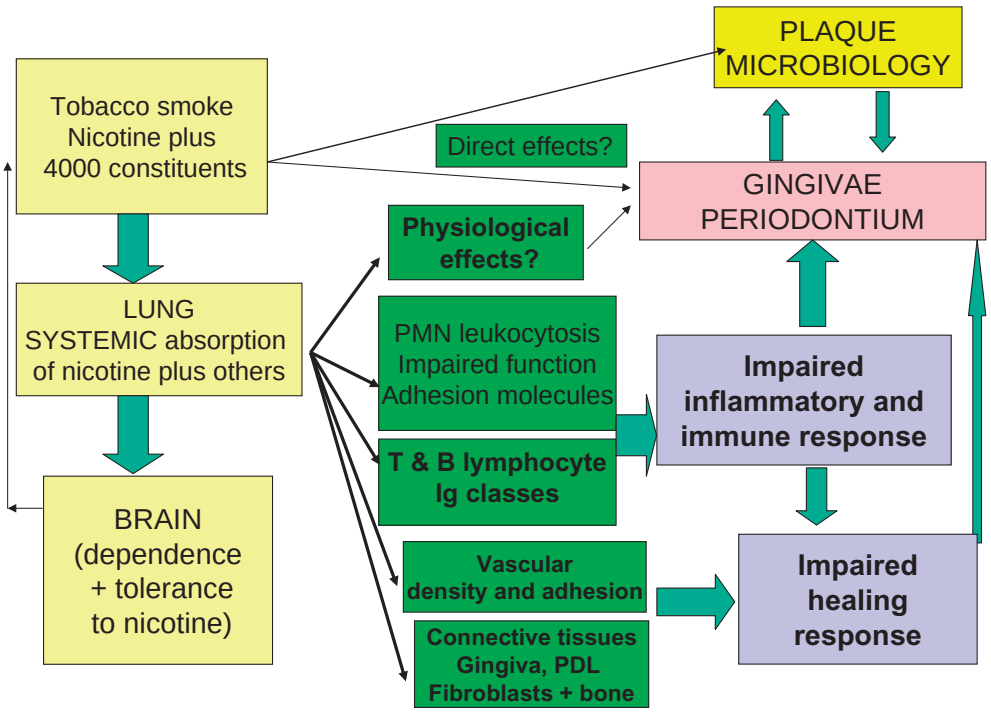

Fig. 12-13 Effects of tobacco smoking on the host response.

advanced attachment loss, and bone loss, as shown in Fig. 12-12b.

Modification of the host–bacteria relationship in smoking

There are several theories as to why smokers have more periodontal disease than non-smokers, involving both bacterial aspects and the host response (Barbour *et al*. 1997; Palmer *et al*. 2005). The potential interactions are illustrated in Fig. 12-13.

Effects on plaque bacteria

Smokers may have higher levels of plaque than non-smokers, which may be accounted for by poorer levels of oral hygiene rather than higher rates of supragingival plaque growth (Bergstrom 1981; Bergstrom & Preber 1986). Several studies have shown that smokers harbor more microbial species which are associated with periodontitis than non-smokers, including *P. gingivalis*, *A. actinomycetemcomitans*, *Tanerella forsythia (Bacteroides forsythus)* (Zambon *et al*. 1996), *P. intermedia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, *Campylobacter rectus* (van Winkelhoff *et al*. 2001), *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Kamma *et al*. 1999). Smokers may have a higher proportion of sites harboring these putative periodontal pathogens, in particular the palatal aspects of the maxillary teeth and the upper and lower incisor regions (Haffajee & Socransky 2001a,b). In contrast several studies have failed to show differences in the bacterial species between smokers and non-smokers (Preber *et al*. 1992; Darby *et al*. 2000; Bostrom *et al*. 2001; van der Velden *et al*. 2003). Microbiological studies differ in their methodology, ability to identify and quantify putative pathogens, and the number of subjects included. Changes in the pocket environment secondary to the effect of smoking on the host tissues could result in a different microflora in smokers.

Effects on the host response

The relationship between plaque accumulation and development of inflammation in smokers has been studied in classical experimental gingivitis studies (Bergstrom & Preber 1986). They demonstrated that there is no difference in plaque accumulation when comparing smokers and non-smokers. However, the development of inflammation was very much retarded in the smoking group with less sites exhibiting redness or bleeding on probing. They also showed lower amounts of gingival crevicular fluid (GCF) during the development of gingivitis. Smoking may result in lower resting GCF flow rate (Persson *et al*. 1999) and an episode of smoking may produce a transient increase in GCF flow rate (McLaughlin *et al*. 1993). The reduced bleeding has previously been proposed to be caused by nicotine-induced vasoconstriction, but as previously described in this chapter, more recent evidence has failed to show a reduction in blood flow to the gingiva following smoking a cigarette in regular smokers (Meekin *et al*. 2000). The reduced bleeding on the other hand is probably due to long-term effects on the inflammatory lesion. Histological comparisons of the lesions from smokers and non-smokers have shown fewer blood vessels in

the inflammatory lesions of smokers (Rezavandi *et al*. 2001). It is pertinent to note that gingival bleeding on probing has been shown to increase within 4–6 weeks of quitting smoking (Nair *et al*. 2003), and this parallels reported recovery of reduced serum ICAM levels (Palmer *et al*. 2002).

Smoking has a profound effect on the immune and inflammatory system (reviewed by Barbour *et al*. 1997; Palmer *et al*. 2005). Smokers have an increased number of leukocytes in the systemic circulation (Sorenson *et al*. 2004), but fewer cells may migrate into the gingival crevice/pocket (Eichel & Shahrik 1969). Smoking is associated with chronic obstructive pulmonary disease (Barnes 2000) and many of the mechanisms indicated are paralleled in findings related to periodontal disease. It is thought that the main cell type responsible for destruction of lung parenchyma is the neutrophil, which is delayed in its transit through the pulmonary vasculature (McNee *et al*. 1989), where it is stimulated to release proteases including elastase, cathepsins, and matrix metalloproteases (Barnes 2000). These destructive molecules are balanced by inhibitors such as α-1-antitrypsin and tissue inhibitors of matrix metalloproteases.

Studies *in vitro* have shown a direct inhibition of neutrophil and monocyte–macrophage defensive functions by high concentrations of nicotine that may be achieved in patients using smokeless tobacco (Pabst *et al*. 1995). MacFarlane and co-workers (1992) examined patients with refractory periodontitis and found a high proportion of smokers in this diagnostic group. These investigators demonstrated abnormal PMN phagocytosis associated with a high level of cigarette smoking.

The PMN is a fundamental defense cell in the periodontal tissue. There is a constant traffic of PMNs from the gingival vasculature through the connective tissue and junctional epithelium into the gingival sulcus/pocket. This is described in some detail in Chapter 11. The PMN is the first line of defense and is chemotactically attracted to bacterial challenge at the dentogingival junction. The PMN contains a powerful battery of enzymes including elastase and other collagenases that have been implicated in tissue destruction in periodontitis and pulmonary disease. Eichel and Shahrik (1969) suggested decreased PMN migration into the oral cavity of smokers. Subsequently, PMNs harvested from the gingival sulcus of smokers were shown to have reduced phagocytic capacity compared to PMNs from non-smokers (Kenney *et al*. 1977). Neutrophil defects have been associated with an increased susceptibility to periodontitis, including cyclic neutropenia where there is a reduction in the number of neutrophils, and conditions such as leukocyte adhesion deficiency (LAD 1 and LAD 2), which may be responsible for cases of generalized prepubertal periodontitis as described by Page *et al*. (1983). It is proposed that smoking causes alterations to PMN function which could be considered to be minor variations of these more profound defects.

The normal passage of the PMN from the microvasculature to the periodontal tissues involves a classic series of events including capture, rolling on the endothelium, firm adhesion to the endothelium, and transmigration through the vessel wall into the connective tissue (Ley 1996). This involves a complex interaction between receptors and ligands on the leukocyte surface and endothelium including selectins, ICAM-1 and LFA1 (CD18, CD11b) (Crawford & Watanabe 1994; Gemmel *et al*. 1994). Defects in the functional ligands for the selectins have been implicated in LAD 2 and mutations in the gene encoding CD18 resulting in absence of the β 2 integrins with LAD 1. Subjects with LAD are susceptible to serious and life-threatening infections and have tremendous destruction of the periodontal tissues, often leading to total tooth loss in the deciduous dentition. These serious and rare conditions illustrate the overwhelming importance of the adhesion molecules and suggest that minor defects in them may also give rise to more subtle conditions that could lead to increased susceptibility to periodontal destruction. In this respect, it has been shown that smokers are affected by upregulation of molecules such as ICAM-1 on the endothelium and they have higher levels of circulating soluble ICAM-1 which could interfere with the normal receptor ligand binding and function of the leukocyte in the defense of the periodontal tissue (Koundouros *et al*. 1996; Palmer *et al*. 1999; Scott *et al*. 2000a). A potential destructive mechanism is the release of elastase from neutrophils following binding of ICAM with CD18 (Mac 1 and LFA 1) (Barnett *et al*. 1996). Lower levels of elastase detected in the gingival fluid of smokers compared to non-smokers, may indicate more elastase release within the tissues (Alavi *et al*. 1995), and this is especially important considering the effects of smoking on protease inhibitors.

Tobacco smoking has a chronic effect on the elevated levels of soluble ICAM (sICAM) and there is evidence that the subject may return to more normal levels after quitting smoking (Scott *et al*. 2000b; Palmer *et al*. 2002). These molecules can be detected in the serum and in the GCF. It has also been shown that cotinine is present in the GCF in about the same concentration as it appears in serum, but the levels of sICAM are much lower in smokers despite very much higher serum levels than non-smokers (Fraser *et al*. 2001). Many other molecules have been studied in the GCF of smokers with many reporting reduced levels compared to non-smokers. Alavi *et al*. (1995) showed significantly lower concentrations of elastase and elastase complexed with α1-antitrypsin in smokers GCF. Although Bostrom *et al*. (1999) showed higher levels of TNF-α in GCF in smokers, they reported no differences in levels of IL-6 (Bostrom *et al*. 2000). Rawlinson *et al*. (2003) found levels of IL-1beta and IL-1ra to be significantly lower in GCF from diseased sites in smokers compared to non-

smokers, and Petropoulos *et al*. (2004) showed that the concentration of IL-1alpha in GCF of smokers was approximately half that found in non-smokers.

PMN-related periodontal tissue destruction may also be related to suppression or exacerbation of the respiratory burst and generation of reactive oxygen species. For example 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 to neutrophils from non-smokers. Thus, inappropriate 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).

The effects of smoking on lymphocyte function and antibody production are very complex, with the various components having the potential to cause immunosuppression or stimulation. It is likely that the particulate phase of cigarette smoke confers immunosuppressive properties. Acute or chronic exposure to hydrocarbons may stimulate or inhibit the immune response, the net effect being dependent upon the dose and duration of the exposure to components of tobacco smoke. The leukocytosis observed in smokers results in increased numbers of circulating T and B lymphocytes (reviewed in Sopori & Kozak 1998). Studies that have examined T cell subsets report different findings of either reduced, increased or no change in the number of CD4 T cells (Loos *et al*. 2004). Smoking appears to affect both B and T cell function, inducing functional unresponsiveness in T cells.

It has been reported that serum IgG levels in smokers may be reduced (Quinn *et al*. 1998) with depression of IgG2, particularly in some racial groups (Quinn *et al*. 1996; Graswinkel *et al*. 2004). Reported levels of serum IgA and IgM classes are variable and IgE may be elevated (Burrows *et al*. 1981).

The clinical change in the tissues of smokers was described above. It is not surprising that histological evaluation of smokers' tissues has shown that there is a decrease in the vascularity of the tissues (Rezavandi *et al*. 2001). This is a chronic effect due to smoking and may also be associated with alterations in the expression of adhesion molecules within the endothelium. The effect of tobacco smoking on the expression of adhesion molecules on leukocytes, within the inflammatory lesion, in the junctional epithelium and cells of the pocket epithelium could have important implications on the progession of periodontitis in smokers. The effect of smoking on macrovasular disease is well documented (Powell 1998) and its effects on microvascular disease could also be of importance in periodontal disease and in healing.

Effects on healing and treatment response

The healing potential of tissues has important implications in any chronic inflammatory lesion and in repair following treatment. Smoking has been identified as an important cause of impaired healing in orthopedic surgery, plastic surgery, dental implant surgery (Bain & Moy 1993), and in all aspects of periodontal treatment including non-surgical treatment, basic periodontal surgery, regenerative periodontal surgery, and mucogingival plastic periodontal surgery (Preber & Bergstrom 1986; Miller 1987; Tonetti *et al*. 1995; Grossi *et al*. 1996, 1997a; Kaldahl *et al*. 1996; Bostrom *et al*. 1998; Tonetti 1998; Kinane & Chestnutt 2000; Heasman *et al*. 2006).

In non-surgical treatment, smoking is associated with poorer reductions in probing depth and gains in clinical attachment. In most studies smokers have a lower level of bleeding at baseline, and following treatment bleeding scores are reduced in smokers in a similar manner to those in non-smokers. The poorer reductions in probing depths and gains in attachment level amount to a mean of approximately 0.5 mm. Much of this may be due to less recession of the marginal tissues in smokers as there is less edema and more fibrosis in the gingiva. The same may be true for the deeper tissues of the periodontium where there is less of an inflammatory infiltrate and vascularity at the depth of the pocket. These differences in the tissues between smokers and non-smokers in the untreated state may largely account for the differences in response to non-surgical treatment. It has been proposed that these differences may be manifest by differences in probe penetration in smokers and non-smokers, particularly in deep pockets (Biddle *et al*. 2001).

The poor response of smokers to non-surgical treatment may also apply to those treated with adjunctive antibiotics (Kinane & Radvar 1997; Palmer *et al*. 1999). Response to non-surgical treatment may be seen merely as resolution of inflammation and improvement of the epithelial attachment together with some formation of collagen. However, the response following periodontal surgery is more complex and involves an initial inflammatory reaction followed by organization of the clot, and formation of granulation tissue consisting of capillary buds and fibroblasts laying down collagen. The surgical flaps have to revascularize and the epithelial attachment has to reform on the surface. In regenerative surgery there also has to be formation of a connective tissue attachment and cementogenesis. Tobacco smoke and nicotine undoubtedly affect the microvasculature, the fibroblasts and connective tissue matrix, the bone and also the root surface itself. It has been shown in *in vitro* studies that fibroblasts are affected by nicotine in that they demonstrate reduced proliferation, reduced migration and matrix production, and poor attachment to surfaces (Raulin *et al*. 1988; Tipton & Dabbous 1995; James *et al*. 1999; Tanur *et al*. 2000). The root surfaces in smokers are additionally contaminated by products of smoking such as nicotine, cotinine, acrolein, and acetaldehyde, and these molecules may affect the attachment of cells (Raulin

et al. 1988; Cattaneo *et al*. 2000; Gamal & Bayomy 2002; Poggi *et al*. 2002). Smoking has a direct effect on bone and is an established risk factor in osteoporosis. It has also been proposed that it may have a direct affect on bone loss in periodontitis (Bergstrom *et al*. 1991) and it undoubtedly delays healing of bone in fracture wound repair. It is not surprising therefore that tobacco smoking has been implicated in poorer responses to periodontal surgical treatment.

Smoking cessation

All patients should be assessed for smoking status and given advice to quit the habit. About 70% of people who smoke would like to quit and should be assisted. They should be referred to specialist cessation services if the treating practitioner does not feel confident in this area. They can be advised about nicotine replacement therapy. People's success with quitting is considerably improved using nicotine replacement therapy and drugs such as buproprion hydrochloride. Former smokers more closely resemble non-smokers in their periodontal health status and response to treatment, but the time required to revert to this status has not been defined. In one of the few papers that have attempted to combine a quit smoking and periodontal treatment interventional study, Preshaw *et al*. (2005) showed a more favorable periodontal treatment outcome in those subjects that managed to quit using well established quit smoking strategies including counseling, nicotine replacement therapy and bupropion. From the original group of 49 subjects there were only 11 continuous quitters at 12 months. It would be of great interest to determine what changes in periodontal status would have occurred with just the quit smoking intervention, and more randomized controlled clinical trials are required in this area.

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