21 Anti-caries strategies

Table 21.1 Microbial stages in the production of carious lesions, the bacterial factors involved and potential strategies for intervention.

Stages	Bacterial factor	Strategy
Adherence	Antigen I/II	Immunization
↓		
Survival and Growth	VikRK ComCDE	TCS inhibitors Natural inhibitors Targeted antibacterial peptides
↓		
Biofilm	Antigen I/II LuxS	Immunization QS inhibitors Photodynamic therapy Probiotics
↓		
Complex plaque	GTF	Immunization Inhibitors
↓		
Accumulation	GTF GBP	Immunization
↓		
Acid	LDH DexA	Probiotics Xylitol
↓		
Dental caries		Fluoride

Table 21.2 Caries immunization strategies

Immunization*	Host	Effect
Killed whole cells of *S. mutans* (parenteral) (A)	Monkey	Protection against *S. mutans* induced caries
Purified surface antigens I/II and WapA (Antigen III) (parenteral) (A)	Rodent Monkey	Protection against *S. mutans* induced caries
Monoclonal antibody to Antigen I/II (oral) (P)	Monkey Human	Prevents colonization or re-colonization by *S. mutans*
Hen-egg antibody to killed whole *S. mutans* cells (digestive tract) (P)	Human	Inhibits colonization by mutans streptococci
IgA Fab antibody to Antigen I/II from transgenic tobacco plants (digestive tract) (P)	Monkey	Inhibits colonization by mutans streptococci
GTF enzymes or fragments (parenteral) (A)	Rodent	Protection against *S. mutans* induced caries
GBP-B	Rodent	Protection against caries
GBP-B and Antigen I/II peptides in liposomes (nasal spray) (A)	Rodent Human	Protection against caries Proposed trials
Antigen I/II N-terminal fragment coupled to CtxB (cholera toxin B subunit adjuvant) (nasal spray) (A)	Human	Trials

*Active (A) immunization depends upon the application of antigen to generate protective antibodies. Passive (P) immunization is the application of pre-formed antibodies.

Dental caries proceeds through a temporal series of events, sometimes taking many months or years. Caries results from the accumulation of acid-producing dental plaque which develops by bacterial adherence to salivary pellicle, formation of a biofilm containing many species of bacteria, and polysaccharide-mediated accumulation of microorganisms. Plaque removal is effectively practiced by tooth brushing with an abrasive paste. However, inter-proximal plaque is difficult to remove, as is subgingival plaque. The route towards dental caries is shown in Table 21.1. The possible targets (bacterial factors) for control strategies to be applied at each of these stages are also shown.

Inhibitors

Over the years, many studies have been undertaken on the effects of inhibitors at several stages of caries development. There have never been any major attempts to utilize antibiotics to control caries although caries rates are lower in patients on long-term antibiotic therapy, such as tetracycline to control chronic acne. Effective natural inhibitors of microbial growth and biofilm formation include polyphenols (from tea extracts) and catechins (from green tea). High molecular mass components present within cranberry juice inhibit biofilm formation. Apigenin, a naturally occurring flavonoid, is a potent inhibitor of GTFs and reduces *S. mutans* biofilm formation. The precise modes of action of these compounds on oral bacteria are not known. Selectively targeted antimicrobial peptides have been generated by fusion of a species-specific targeting peptide domain (CSP in the case of *S. mutans*) with a wide-spectrum antimicrobial peptide domain (derived from novispirin G10). Xylitol is an artificial sweetener that is taken up by *S. mutans* cells, via a fructose transporter and inhibits glycolysis. With the realization that bacteria are able to communicate with each other, and that this is essential for biofilm formation, synthetic inhibitors have been designed that interfere with signal recognition by two-component signal transduction systems (TCS), such as VikKR, or mimic the signaling molecules, thus interfering with QS quorum sensing or LuxS/AI-2 communication. Lastly, photodynamic therapy has been proposed as an effective means to control plaque formation. A chemical photosensitizer, such as toluidine blue, is allowed to become incorporated into biofilms. Bacteria are then killed by irradiation with red light.

Probiotics

Probiotic therapy provides a live microbial supplement that beneficially affects the host's healthy microbial balance. Probiotics have commonly been associated with food supplements, such as *Lactobacillus* and *Bifidobacterium* within yogurts. *Lactobacillus* species have also been shown to have some effect on preventing oral colonization by *S. mutans*. A novel approach to biological control of *S. mutans* has been to engineer a strain that lacks lactate dehydrogenase (does not produce lactic acid) and produces a potent bacteriocin that kills other *S. mutans*. In theory, introduction of this strain into the mouth, will destroy other *S. mutans* but reside harmlessly in plaque unable to produce lactic acid.

Immunization

The generation of antibodies to bacteria, or bacterial virulence factors, has been central to the control of many infections. Immunization may be passive (the application of preformed antibodies) or active (vaccination with antigens to generate antibodies). Passive immunization regimens have been very promising for control of *S. mutans*, but none has yet been brought into practice (Table 21.2). Vaccination of cows or hens with GTF or GBP preparations (see Chapter 16) led to the production of high-titer antibodies to these proteins in milk or eggs. The milk antibodies were found to be very effective at preventing *S. mutans* colonization in animal studies. Antibodies generated in mice to AgI/II were found to be effective in preventing *S. mutans* induced caries in animals. These studies have been extended to producing S-IgA to AgI/II in transgenic plants (plantibodies). The AgI/II antibodies, when applied to professionally cleaned teeth of volunteers, inhibited the re-emergence of *S. mutans* for over a year.

Vaccination

Studies in the 1970s showed that vaccination of laboratory animals with killed cells of *S. mutans* generated antibodies that protected against dental caries. Since then a large body of work has concentrated on developing a potential vaccine for humans. There were many fears of generating autoimmune responses with these whole cell vaccines. Consequently, there has been more emphasis on designing acellular vaccines comprised of surface proteins or antigenic segments of these, or of DNA. Fusion of immunogens to the B sub-unit of cholera toxin as a mucosal adjuvant improves antigen specific memory responses to oral vaccines. Expression of recombinant *S. mutans* antigens in attenuated strains of *Salmonella* is another approach to boost antibody responses. One of the most promising vaccines to date contains small segments of the AgI/II and GbpB linked together. The suggestion is that this might be utilized to vaccinate young children who have been identified as being especially prone to caries. The vaccine could be administered as a nasal spray containing an adjuvant, which stimulates immune cell responses. As such, it has been shown that this generates IgG circulatory antibodies, as well as mucosal IgA antibodies. The idea is that this method of vaccination may induce protection at the oral surface against colonization (through IgA), and provide IgG antibodies that reach the oral cavity following secretion in gingival crevicular fluid.

22 Periodontal diseases

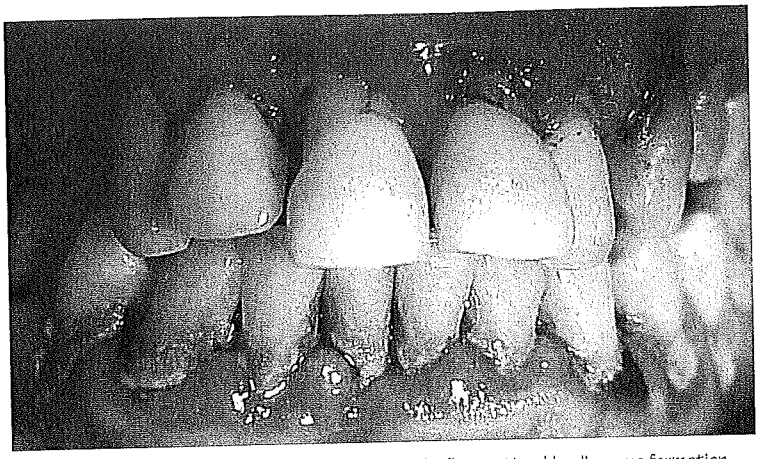

Figure 22.1 Severe chronic periodontitis with gingival inflammation, bleeding, pus formation and tissue destruction.

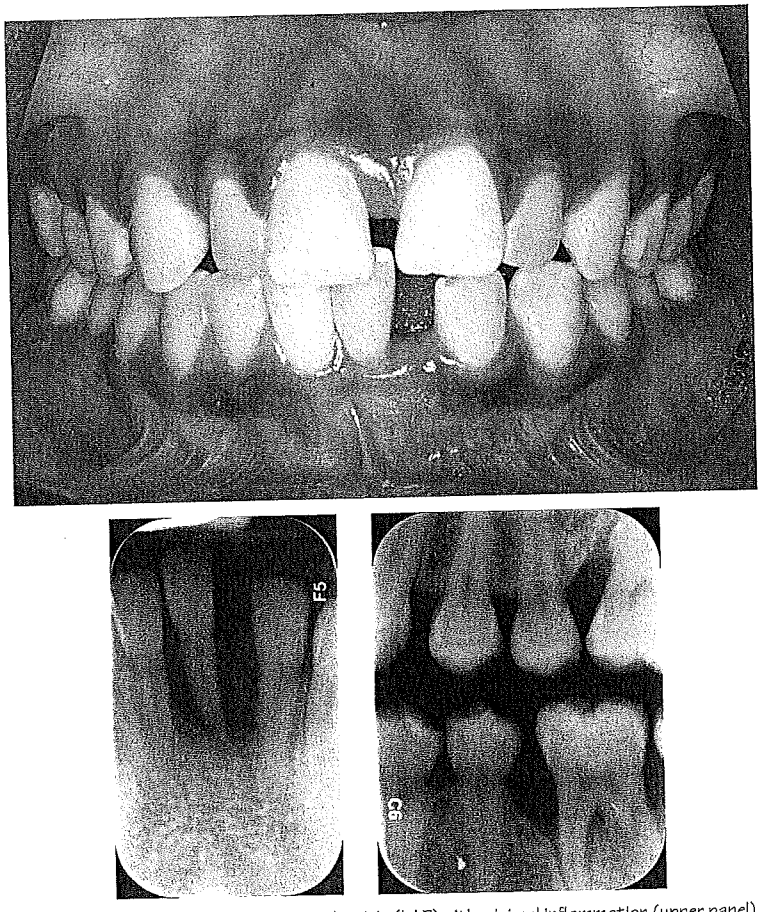

Figure 22.2 Localized aggressive periodontitis (LAP) with minimal inflammation (upper panel), but bone loss around incisors and first permanent molars is evident on X-ray (lower panels).

Bacteria in combination with the host inflammatory response are responsible for most forms of periodontal disease. Periodontal diseases are mixed infections and a variety of bacteria or groups of bacteria are required for the initiation and progression of the disease in a susceptible host. The identities of these organisms can vary among diseases, among patients and even among sites in the same patient. These bacteria are often present in healthy individuals, and thus periodontal diseases can be considered opportunistic infections.

The development of periodontal disease is associated with deepening of the gingival crevice into a periodontal pocket that can be several millimeters in depth and bleeds upon probing. Periodontal diseases are very common and it is estimated that over 50% of adults in the USA have experienced some form of the disease, although less than 10% have the severe forms.

Classification of periodontal diseases

There are many different types of periodontal disease. Some of the most common are:

(1) **Gingivitis** Gingivitis is generally the result of overgrowth of supragingival plaque that irritates the gingival tissues. Gingivitis is a reversible inflammation that can be exacerbated by systemic factors, e.g. pregnancy, viral immunosuppression.

(2) **Periodontitis** Periodontitis is an inflammatory-based infection of the supporting structures of the teeth, with progressive destruction of the periodontal ligament and alveolar bone, leading to tooth loss. Periodontitis is the most common cause of adult tooth loss in developed countries. There are two major forms: chronic and aggressive.

(a) **Chronic periodontitis** This is the most common manifestation, and usually occurs in adults (Figure 22.1). The severity is consistent with local factors of plaque and calculus. It is episodic, with overall slow progression, and can be exacerbated by systemic factors.

(b) **Aggressive periodontitis** In this case severity is not consistent with local factors of plaque and calculus. There is rapid tissue destruction. A strong genetic component is indicated by the familial pattern of occurrence and differing incidences in ethnic groups (there is a 15-fold higher incidence of LAP in African-American populations as compared to other groups). Aggressive periodontitis can be further subdivided:

(i) Localized aggressive periodontitis (LAP), formerly known as localized juvenile periodontitis (LJP). The onset of LAP is around puberty, and the disease is restricted to the incisors and first molars. Usually there is little inflammation and individuals may not realize they have the disease until given an X-ray at a later date (Figure 22.2).

(ii) Generalized aggressive periodontitis (GAP). This manifestation is found in patients under 30, and involves at least three teeth other than incisors and first molars.

(3) **Necrotizing periodontal diseases** These are characterized by painful necrosis of gingival tissues, periodontal ligament and alveolar bone. The disease is often associated with emotional stress (the condition was called "trench mouth" among soldiers in World War I) and with systemic conditions including HIV infection, malnutrition and immunosuppression.

(4) **Peri-implantitis** As the name suggests, this is destruction of tissue and bone surrounding an implant, and similar to chronic periodontitis.

Role of plaque bacteria in periodontal diseases

The metabolic action of early bacterial colonizers in the gingival crevice alters the environment and facilitates colonization by secondary organisms. These secondary colonizers tend to be more pathogenic and when they exceed threshold levels disease can occur. However, the mere presence of periodontopathic bacteria does not necessarily result in disease. The concordance of a variety of bacterial virulence factors, the activity and composition of the commensal microbiota, and host immune factors, are required for the initiation of the disease process (see the Ecological plaque hypothesis in Chapter 9). Periodontal pathogens are discussed in more detail in succeeding chapters.

Role of host factors in periodontal diseases

As with many infections, the pathology associated with periodontal diseases may be caused by immunopathogenic mechanisms. This is discussed further in Chapter 26. Other host factors that can adversely affect periodontal health are: smoking/tobacco use, which disrupts immune function and limits local blood flow; genetics (Chapter 26); pregnancy/puberty, when there are changes in the levels of hormones that can affect the immune response and can be used for nutrition by some pathogenic bacteria; systemic diseases such as diabetes, stress, obesity; and poor nutrition that can affect host immune status. As discussed in Chapter 26, neutrophils are important as a first line of defense against periodontal bacteria, and susceptibility to periodontal disease often correlates with neutrophil deficiencies. Antibodies to periodontal bacteria are also found in serum and in gingival crevicular fluid (GCF) in most forms of the disease, although there is usually a poor antibody response in GAP. The protective function of antibodies in the GCF mainly relates to opsonization and increased phagocytosis. Antibodies to *A. actinomycetemcomitans* (Chapter 23), the predominant pathogen in LAP, may be one reason why the disease is limited to the incisors and first permanent molars. These teeth are the first to erupt into the oral cavity and after infection subsequent antibody production may prevent infection of later erupted teeth and the disease "burns out". Developmental or acquired anatomical deformities can predispose to periodontal disease, particularly those relating to gingival excess that create an environment conducive to the retention of bacteria in the periodontal area.

23 Microorganisms associated with periodontal diseases

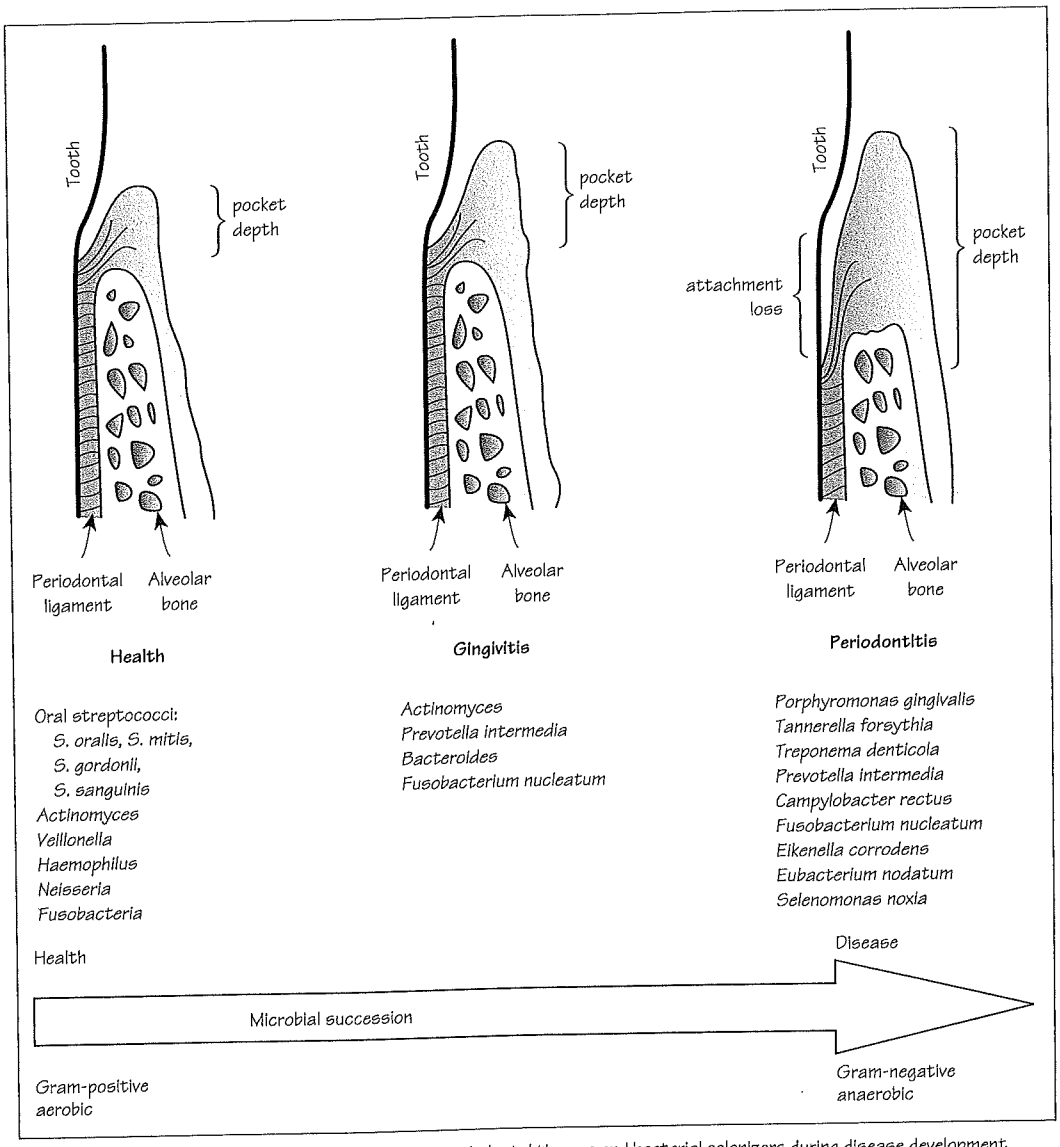

Figure 23.1 Schematic representations of changes in periodontal tissues and bacterial colonizers during disease development. Reproduced in modified form with permission from Lamont RJ, Burne RA, Lantz MS, LeBlanc DJ (eds) *Oral Microbiology and Immunology* (2006) ASM Press, Washington DC.

As discussed in Chapter 22, specific groupings of bacteria are associated with the initiation and progression of different types of periodontal disease (Figure 23.1). The association of these organisms with disease is based on several criteria: elevated numbers in disease compared to health; reduction in numbers following therapy; host responses to the organism in disease; pathogenicity in animal models; and presence of relevant virulence factors. While none of these alone is definitive, the concordance of a number of such attributes increases the likelihood that the organism is a pathogen. It is also important to remember that most of the putative pathogens are also present in healthy mouths and disease requires a shift in the balance between host and organism that allows the pathogenic bacteria to increase in number and express their virulence factors at susceptible sites (Chapter 25).

Gingivitis

The organisms involved in gingivitis tend to also be commonly found in mature supragingival plaque in healthy individuals.

Actinomyces spp: Gram-positive facultatives that comprise a large proportion of the supragingival and subgingival plaque microbiota.

Prevotella intermedia: a Gram-negative black pigmenting anaerobe.

Bacteroides species: Gram-negative anaerobes.

Fusobacterium nucleatum: a Gram-negative anaerobe present in high numbers in supra- and sub-gingival plaque both in health and disease.

Chronic periodontitis

Organisms associated with chronic periodontitis are secondary, often anaerobic, colonizers of mature subgingival plaque that possess tissue destructive properties.

Porphyromonas gingivalis: a highly proteinaceous, asaccharolytic, Gram-negative black pigmenting anaerobe.

Tannerella forsythia: a Gram-negative anaerobe with an outer S-layer.

Treponema denticola and other medium and large size spirochetes. Spirochetes are corkscrew shaped, generally difficult to culture, and are motile by means of their axial filaments/endoflagella.

Prevotella intermedia: see above.

Campylobacter rectus: Gram-negative, motile, anaerobe.

Fusobacterium nucleatum: see above. Different strains or subspecies may exhibit different virulence.

Eikenella corrodens: a Gram-negative facultative. Colonies form pits on agar, hence the name 'corrodens'.

Eubacterium nodatum: a Gram-positive anaerobe.

Selenomonas noxia: Gram-negative, curved rod. Exhibits tumbling motility and has a tuft of flagella in the concave side.

Peptostreptococci: Gram-positive anaerobic cocci.

Herpes viruses: may work synergistically with bacteria.

Localized aggressive periodontitis

LAP is almost exclusively associated with *Aggregatibacter actinomycetemcomitans*, a Gram-negative capnophile.

Generalized aggressive periodontitis

GAP is associated with a subset of organisms involved in chronic periodontitis: *P. gingivalis*, *Tannerella forsythia*, *P. intermedia*, *P. nigrescens* (closely related to *P. intermedia*) and *Selenomonas*.

Color-coded complexes

In the plaque biofilm, groups of metabolically compatible organisms assemble into complexes that have distinct pathogenic potential. It can be instructive, therefore, to color code these complexes according to their potential danger to the host. The most pathogenic of these groupings is the red complex, comprising *P. gingivalis*, *T. forsythia* and *T. denticola*. The complexes also become spatially and temporally associated in developing subgingival plaque.

P. gingivalis, a consensus pathogen

While there is considerable debate over the relative pathogenic status of many oral organisms, most in the field would agree that *P. gingivalis* is among the most pathogenic. *P. gingivalis* is a Gram-negative anaerobic coccobacillus, closely related to the *Bacteroides*, although the organism is fairly aereotolerant and can even grow under low oxygen conditions. Aerotolerance presumably aids transmission of the organism between individuals and early survival in the oral cavity. *P. gingivalis* is asaccharolytic, meaning that it relies on the catabolism of amino acids for energy production and growth. Degradation of proteins and peptides is thus important for the *P. gingivalis* nutrition, and the organisms express a number of proteolytic enzymes with differing specificities. *P. gingivalis* preferentially acquires iron in the form of heme (a ferrous complex found in hemoglobin and other host proteins). The availability of heme regulates virulence expression by the organism (Chapter 25). Heme derivatives accumulate on the bacterial surface, imparting the black pigmented appearance of colonies on blood agar, and help protect against oxidative stress. Indeed, *P. gingivalis* has multiple systems to protect against oxidative stress, including superoxide dismutase, alkyl hydroperoxide reductase, and rubrerythrin. As *P. gingivalis* strains are frequently present in the mouth, regardless of disease status (although in lower numbers in health), *P. gingivalis* is more accurately an opportunistic pathogen. No one clonal type is associated with disease (reflective of a long-term evolutionary relationship between host and micro-organism), although differences in fimbrial type and capsule expression may alter virulence.

A. actinomycetemcomitans, a pathogen in LAP

LAP is almost exclusively associated with *A. actinomycetemcomitans*, a Gram-negative capnophilic (preferring elevated CO_2) coccobacillus. *A. actinomycetemcomitans* is in the *Pasteurellaceae* family, and a member of the HACEK group of pathogens (together with *Haemophilus*, *Cardiobacterium*, *Eikenella* and *Kingella*). Recent clinical isolates tend to be fimbriated (resulting in colonies with a star shape in the center), but production of fimbriae is lost on laboratory sub-culture. Six serotypes (a–f) of the LPS O-polysaccharide are recognized currently, of which serotype b is more commonly associated with disease.

Culture independent bacterial detection

Recent advances in molecular based identification methods have allowed the detection of large numbers of organisms that cannot as yet be cultivated (Chapter 10), and organisms difficult to isolate such as methanogenic Archaea and sulfate-reducing bacteria. Some of these, e.g. the genera *Megasphaera* and *Desulfobulbus*, and *Filifactor alocis* are more abundant in periodontal lesions; however, the pathogenic status of these organisms remain to be established.

24 Colonization by periodontal bacteria

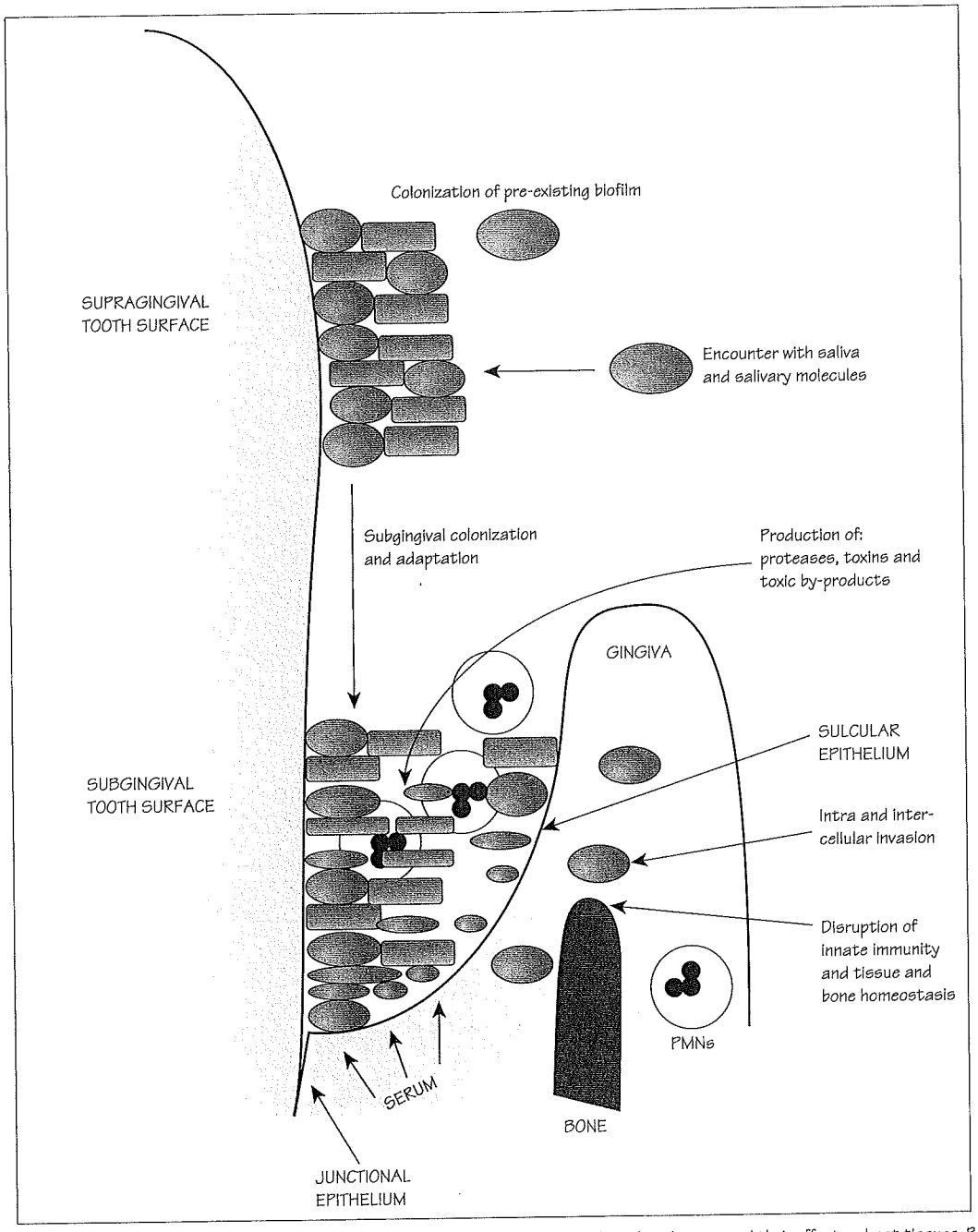

Figure 24.1 Micro-environments in the mouth that are encountered during colonization by periodontal pathogens and their effect on host tissues. Reproduced with permission from Lamont RJ, Burne RA, Lantz MS, LeBlanc DJ (eds) *Oral Microbiology and Immunology* (2006) ASM Press, Washington DC.

Oral bacteria are acquired shortly after birth, usually from a care giver, and transmission through close contact continues throughout life. Motile organisms such as spirochetes can be chemotactically attracted to compounds in the gingival crevice. Non-motile organisms adhere to available surfaces and then reach the subgingival crevice by spreading proliferation from the supragingival surfaces or translocation of dislodged progeny if at a remote site (Figure 24.1).

Adhesion

Periodontal pathogens tend to be later colonizers and adhere to antecedent colonizers and their products. Periodontal bacteria also adhere to the oral soft tissues, either to the epithelial cells directly or to the extracellular matrix.

Periodontal bacterial adhesins

P. gingivalis possesses two (at least) distinct fimbriae. The long fimbriae are comprised of the FimA structural protein and mediate attachment to salivary proline-rich proteins and statherin; early colonizers such as *S. gordonii*; epithelial cells, endothelial cells and fibroblasts; and matrix proteins such as fibronectin and fibrinogen. Many of these binding properties have been located to discrete linear domains in the FimA sub-unit. The long fimbriae are classified into six genotypes (I to V and Ib) on the basis of the diversity of the *fimA* genes. The shorter fimbriae are comprised of the Mfa protein and mediate attachment to other bacteria. *P. gingivalis* expresses two proteins of the Internalin J class of leucine rich repeat (LRR) proteins that are involved in adhesion and biofilm formation. *P. gingivalis* also produces a series of hemagglutinins that bind to host cells, and proteinases (Chapter 25) that possess hemagglutinin domains that can also be involved in adhesion.

A. actinomycetemcomitans possesses the type IV Flp fimbriae that are responsible for non-specific adherence and biofilm formation on solid surfaces, including saliva coated surfaces. The Flp fimbriae are assembled and secreted through the activities of *tad* gene products, a locus that is widespread in other bacteria and archaea. The *tad* locus is on a genomic island and contains 14 genes necessary for the biogenesis of the fimbriae. The Flp fimbriae are readily lost on laboratory subculture and the colonies change from a rough to a smooth appearance. Specific adhesion of *A. actinomycetemcomitans* to epithelial cells is mediated by the autotransporter proteins Aae and ApiA. The extracellular matrix protein adhesin A (EmaA), also an autotransporter, mediates binding to collagen.

An important adhesin of *T. denticola* is a major outer membrane protein Msp, which mediates attachment to matrix proteins and cells. Msp is also a porin and can become integrated into the membranes of host cells with resultant cytotoxicity. In conjunction with proteases (discussed below) Msp degrades the integrity of the periodontium and allows *T. denticola* to invade periodontal tissues.

The RadD outer membrane protein is responsible for arginine-inhibitable adherence of *F. nucleatum* and contributes to coadhesion with other oral bacteria and multispecies biofilm formation. The leucine rich adhesin FadA is responsible for epithelial cell attachment and entry by *F. nucleatum*. FadA is anchored in the inner membrane and protrudes through the outer membrane.

T. forsythia has yet to be investigated in as much detail as some of the other periodontal pathogens. A major adhesin is BspA, a member of the LRR protein family that mediates adherence to host cells and matrix proteins, and to other bacteria. BspA is also required for *T. forsythia* induced bone loss in animals and for entry into epithelial cells.

Intracellular invasion

Adhesion to host cells can be a prelude to internalization. In non-professional phagocytes such as epithelial cells this is a bacterially driven process. Epithelial cells recovered from the oral cavity can contain large numbers of bacteria, indicating that this is an important *in vivo* process.

Mechanisms of invasion

Invasion of *P. gingivalis* is initiated by the interaction of the FimA fimbriae with integrin receptors on gingival epithelial cells. Integrin-dependent signaling, along with signaling induced by a secreted *P. gingivalis* serine phosphatase (SerB), results in remodeling of the host microfilament and microtubule cytoskeleton that is necessary for bacterial engulfment through lipid raft components. Subversion of host cell signal transduction through phosphorylation/dephosphorylation of proteins and calcium ion fluxes allows trafficking of *P. gingivalis* to the perinuclear area and ultimately impacts gene expression in the host cells. Internalized *P. gingivalis* remain viable and undergo major shifts in physiological status, with around 40% of the expressed proteome differentially regulated as the organism adapts to an intracellular environment. *P. gingivalis* suppresses apoptotic cell death through inhibition of intrinsic (mitochondrial) apoptotic pathways, and accelerates progression of epithelial cells through the cell cycle. *P. gingivalis* can spread to uninfected cells through actin based intercellular protrusions and thus prevent removal from the tissue following host cell death and sloughing.

Host responses to intracellular bacteria

Epithelial cells respond to internalized bacteria through gene expression changes that are tailored to the infecting organism. These can have consequences for innate immunity, e.g. *P. gingivalis* suppresses expression of the neutrophil chemokine Interleukin (IL)-8, which will compromise recruitment of neutrophils and also innate immune surveillance (a phenomenon known as localized chemokine paralysis). In contrast, *A. actinomycetemcomitans* is more visible to the innate immune system and induces expression of IL-8, IL-6 and IL-1β from epithelial cells. Such differences in host epithelial cell responses may contribute to the different clinical outcomes associated with these organisms.

25 Virulence factors of periodontal bacteria

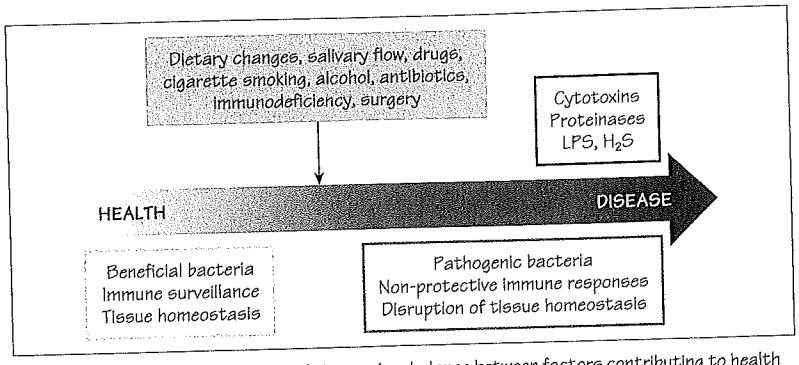

Figure 25.1 The status of periodontal tissues is a balance between factors contributing to health (left side) and factors contributing to disease (right side). External influences influence the flow from health towards disease.

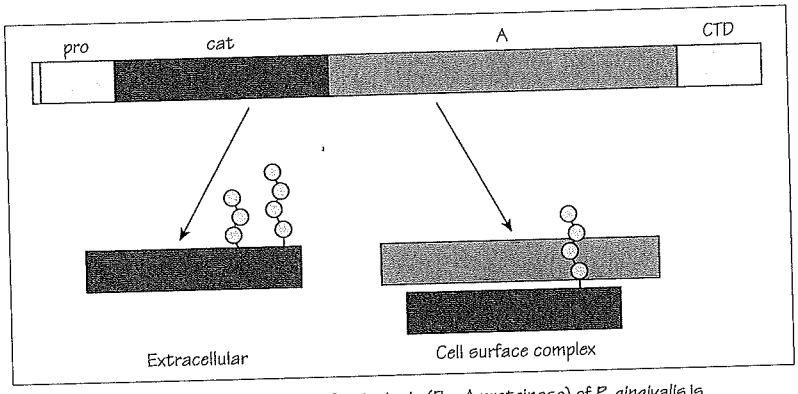

Figure 25.2 The major arginine (R) specific gingipain (RgpA proteinase) of *P. gingivalis* is expressed as a polyprotein comprised of the preprofragment (pro), the catalytic domain (cat), the adhesin/hemagglutinin domain (A) and the C-terminal domain (CTD) responsible for outer membrane translocation. The enzyme can be proteolytically processed into these domains and also processed within the A domain. Enzyme fragments can associate noncovalently in a variety of configurations and can be cell bound or extracellular. The protein is post translationally modified with glycan chains (green circles).

Table 25.1 Major virulence factors of common periodontal pathogens.

Virulence factor	Organism(s)	Action(s)
Leukotoxin	*A. actinomycetemcomitans*	Kills monocytes, neutrophils, and lymphocytes
Cytolethal distending toxin (CDT)		G2 arrest and apoptosis of lymphocytes
Proteases	*P. gingivalis, P. intermedia, T. denticola, T. forsythia*	Degrade matrix components, host cell receptors and immune effector molecules; alter vascular permeability; process bacteria surface adhesins; activate host MMPs, provide substrates for bacterial growth
LPS	Gram-negatives	Induction of inflammatory responses; osteoclast stimulation and bone resorption
Butyric acid and other short chain volatile fatty acids	*P. gingivalis* and other anaerobes	Host cell toxicity
Volatile sulfur compounds, ammonia and indole	*P. gingivalis* and other anaerobes	Host cell toxicity

Periodontal pathogens produce virulence factors that impact host immune responses, impinge upon tissue integrity and disrupt bone homeostasis (see Table 25.1). However, the onset of disease is determined by host factors and external influences, in addition to the periodontal microbiota (Figure 25.1).

Toxins

Periodontal pathogens generally do not produce potent exotoxins, although *Aggregatibacter actinomycetemcomitans* provides an exception. *A. actinomycetemcomitans* produces two major extracellular toxins, leukotoxin (LT) and cytolethal distending toxin (CDT). LT is a member of the RTX (repeats in toxin) family of pore forming hemolysins/leukotoxins expressed by a variety of pathogens. The leukotoxin operon consists of four genes involved in activation and transport of LT. LT targets only monocytes, neutrophils and a subset of lymphocytes from humans and some non-human primates that express the β2-integrin LFA-1. Cell death results from pore formation (high LT doses) or by apoptosis through mitochondrial perturbation (low LT doses). Strains of *A. actinomycetemcomitans* that are isolated from LAP lesions often have a re-arrangement in the promoter region of the LT gene that causes increased LT expression.

CDT is a heat labile cytotoxin homologous to toxins in *Escherichia coli* and some other Gram-negative pathogens. CDT induces G2 arrest of proliferating lymphocytes, as well as eukaryotic cell distension, actin rearrangements and apoptosis. CDT is a heterotrimer and the active sub-unit, CdtB, enters the cell while CdtA and CdtC remain associated with the cell surface.

Proteolytic enzymes

P. gingivalis produces a number of proteases with different specificities, although the best characterized are RgpA (Figure 25.2) and RgpB (arginine specific), and Kgp (lysine specific), collectively known as the gingipains and key virulence determinants. The primary function of *P. gingivalis* proteases is to provide peptides and heme (from host heme sequestering proteins) for the nutrition of this asaccharolytic, largely heme-dependent, organism. However, the broad specificity of these enzymes allows targeting of host structural proteins (e.g. collagens, fibronectin and laminin) and immune effector proteins (e.g. cytokines, antibodies, complement components, antimicrobial peptides and leukocyte surface receptors). Gingipains can activate the kallikrein cascade and cause the release of kinins with subsequent induction of vascular permeability which may allow systemic dissemination of *P. gingivalis*.

Gingipains also activate host matrix metalloproteinases (MMPs), particularly MMP-2 (gelatinase A-type IV collagenase), MMP-8 (neutrophil collagenase-2) and MMP-9 (gelatinase B-type IV collagenase). MMPs are a family of zinc-dependent proteinases secreted by many cell types that are activated by proteolysis and can degrade and modify matrix and basement membrane proteins in the periodontium. MMPs thus contribute to periodontal tissue destruction and failure of the periodontal lesion to heal. Gingipains can also degrade tissue inhibitors of MMPs (TIMPs).

RgpA and Kgp possess hemagglutinin (HagA) domains that mediate attachment to host cells. In addition, some *P. gingivalis* surface molecules such as FimA require protease activity for post-translational processing. Moreover, gingipain activity can expose previously hidden adhesin binding domains (cryptitopes) on host and bacterial proteins. *P. gingivalis* releases large numbers of membrane vesicles by evagination of the outer membrane. These vesicles possess adhesins and LPS present on the bacterial outer membrane, as well as entrapped periplasmic components. As vesicles contain proteases, one function may be to deliver proteolytic enzymes to the gingival tissues, which can also be penetrated by vesicles due to their small size.

Other proteinases produced by *P. gingivalis* include another group of cysteine proteases, aminopeptidases, prolyl dipeptidyl peptidase IV, and an endopeptidase with homology to endothelin-converting enzyme-like endopeptidase (PepO), important for epithelial cell invasion.

Prevotella intermedia produces several proteinases including a cysteine protease designated interpain A, trypsin-like serine proteases, and a dipeptidyl peptidase IV.

Treponema denticola produces a chymotrypsin-like protease CTLP, and a prolylalanine-specific protease PrtP. These proteases can form a complex along with Msp, a major OM protein with adhesive, porin and cytotoxic functions.

Tannerella forsythia produces an arginine specific cysteine protease (PrtH).

Bacterial components that impact alveolar bone

Loss of the alveolar bone that supports the tooth occurs in advanced periodontitis. Bacterial surface components can interfere with the balance between bone deposition by osteoblasts and resorption by osteoclasts. *P. gingivalis* LPS and fimbriae can stimulate osteoclasts and also induce the release from other cells of Interleukin (IL)-1β, PGE2 and TNFα, all of which are mediators of bone resorption. *A. actinomycetemcomitans* can activate osteoclasts through LPS and the molecular chaperone GroEL. Bone loss is discussed further in Chapter 26.

Other toxic products

P. gingivalis and other anaerobes produce butyric acid and other volatile fatty acids as metabolic end products. These short chain fatty acids are cytotoxic and can induce DNA fragmentation and apoptosis. Volatile sulfur compounds (hydrogen sulfide, methyl mercaptan and dimethyl sulfide), ammonia and indole produced by *P. gingivalis* and other anaerobes are also cytotoxic.

26 The immune response and periodontal bacteria

Table 26.1 Characteristics of major cytokines with relevance to periodontal disease

Cytokine	Cell source	Major biological activities
IL-1 α and β	Many cell types	Multiple pro-inflammatory functions, including stimulation of bone resorption, and MMP synthesis and release
IL-1ra	Macrophages, endothelial cells, keratinocytes	Receptor antagonist that inhibits action of IL-1
IL-4	Th2 lymphocytes	B cell proliferation, macrophage inhibition
IL-6	Many cell types	Multiple pro-inflammatory functions, similar to IL-1
IL-8	Macrophages, fibroblasts, PMNs, keratinocytes	Chemotactic for PMNs, T-cells and monocytes
IL-10	CD4/CD8 T cells, monocytes	Anti-inflammatory, inhibits macrophages and T-cells
IL-12	Macrophages, T and B cells	Stimulates Th1 lymphocytes
IL-13	T cells	B cell proliferation, macrophage inhibition
IL-17	T cells	Pro-inflammatory, stimulates bone resorption
TNF α and β	Macrophages, monocytes	Pro-inflammatory and can cause cell death
IFN-γ	NK cell and cytotoxic T-cells	Macrophage activation
MCP-1	Macrophages, monocytes, fibroblasts	Chemotactic for monocytes, T-cells and dendritic cells
MIP-1 α and β	Monocytes, fibroblasts, lymphocytes	Chemotactic for monocytes, T-cells and PMNs

Table 26.2 *Genetic polymorphisms associated with periodontitis or periodontal health. Reproduced with permission from Lamont, Burne, Lantz, LeBlanc (eds) Oral Microbiology and Immunology (2006) ASM Press.*

Polymorphism	Gene	Disease association
IL-1A (+4845) and IL-1B (+3954)	Interleukin-1 gene	Chronic periodontitis
TNF-alpha-308 allele 1	TNF-α gene	Chronic periodontitis
TNF-beta NcoI, ET-1 gene, and ACE gene insertion/deletion polymorphism	Lymphotoxin alpha (TNF-β), endothelin-1 (ET-1) and angiotensin-converting enzyme (ACE) genes	Chronic periodontitis
Fc gammaRIIIb-NA2 allotype	Fc receptor polymorphism	Chronic periodontitis
NAT2	N-acetyltransferase polymorphism	Chronic periodontitis
MMP-1 promoter polymorphism	Matrix metalloproteinase-1 gene	Chronic periodontitis
IL-1A (+4845) and IL-1B (+3954)	Interleukin-1 gene	Aggressive periodontitis
IL-1RN	Interleukin-1 receptor antagonist gene	Aggressive periodontitis (localized)
IL-4 promoter and intron polymorphisms	Interleukin-4 gene	Aggressive periodontitis
FcgammaRIIIb-NA2 allele (and possibly FcgammaRIIIa-158F)	Fc receptor gene polymorphisms	Aggressive periodontitis (generalized)
Gc locus chrom 4q	Unknown	Aggressive periodontitis (localized)
fMLP receptor	N-formyl peptide receptor polymorphisms	Aggressive periodontitis (localized)
VDR ApaI polymorphism	Vitamin D receptor polymorphism	Chronic and aggressive periodontitis (localized)
VDR gene *Taq* 1 polymorphism	Vitamin D receptor polymorphism	Aggressive periodontitis
HLA-A28 and HLA-B5	HLA haplotype	Periodontal health
FcgammaRIIIb-NA1	Fc receptor polymorphisms	Periodontal health

The initial periodontal lesion is characterized by an acute inflammatory response with vascular changes, collagen degradation and neutrophil infiltration. The host immune response is a double-edged sword that can be protective or destructive depending on context. A T-helper (Th)1 response develops in the early lesion with an increase in T lymphocyte infiltration, elevated loss of collagen and migration of epithelium down the root surface. However, interleukin (IL)-12 (Table 26.1) is produced which induces IFN-γ, which in turn activates macrophages and contributes to control of bacterial overgrowth. As the lesion becomes established, the levels of B lymphocytes, along with neutrophils, plasma cells and monocytes, begin to increase. Established and advanced lesions are consistent with Th2 responses, with production of IL-4, IL-10 and IL-13 and consequent antibody production. If the Th2 responses are insufficient to resolve or control the lesion there is subsequent additional connective tissue loss and osteoclastic alveolar bone loss. Recently, a novel subset of T-helper (Th) cells was identified that secretes several proinflammatory cytokines, including IL-17. IL-17 can support Th1 responses and promote neutrophil recruitment (protective responses) but may also stimulate osteoclastic bone resorption in combination with RANK-L (see below).

Importance of neutrophils

Neutrophils recruited through the gingival tissues create a barrier between the junctional epithelium and bacteria in the gingival crevice, and are thus the first line of innate defense against periodontal pathogens. Severe periodontitis, especially in young children, is frequently associated with congenital diseases that involve defects in neutrophil function such as leukocyte adhesion deficiency, Chediak–Higashi syndrome, Papillon–Lefèvre syndrome and chronic/cyclic neutropenia.

Not surprisingly, successful periodontal pathogens have evolved strategies to avoid neutrophil killing. *P. gingivalis* produces capsular polysaccharide that confers resistance to phagocytosis. Proteolytic degradation of opsonins (antibody and complement components) and neutrophil receptors by *P. gingivalis* also impedes phagocytosis. *P. gingivalis* can antagonize IL-8 secretion by epithelial cells following stimulation by commensal organisms, thus decreasing recruitment of neutrophils into the gingival crevice. In addition, neutrophils that remain in the gingival tissues and encounter bacteria can disgorge their lytic enzymes and contribute to tissue damage. *A. actinomycetemcomitans* produces Fc-binding proteins that inhibit phagocytosis by competing with neutrophils for binding to antibody opsonins. Once ingested, periodontal pathogens also show resistance to intracellular killing.

Innate immune sensing of periodontal bacteria

Cells of the innate host defense recognize periodontal bacteria, but there are mechanistic differences compared to commensal recognition, and a gradation of responses that may impact pathogenic potential. Conserved bacterial structures (microbe associated molecular patterns, MAMPs), such as LPS are recognized by host cell pattern recognition receptors (PRRs), such as the toll-like receptors (TLRs), that initiate signaling events which control production of immune effector molecules such as cytokines (Chapter 8). The LPS from *P. gingivalis* is unusual in that it does not elicit potent inflammatory reactions in mice and can signal through both TLR2 and TLR4. *P. gingivalis* LPS displays lipid A structural heterogeneity, containing both penta and tetra-acylated lipid A structures, the ratios of which vary according to the concentration of hemin in the growth medium. The penta-acylated lipid A structures are TLR4 agonists, whereas tetra-acylated structures are TLR4 antagonists. The FimA fimbriae of *P. gingivalis* signal through TLR2 and TLR4, although other PRRs, such as CD14 and CD11b/CD18, are involved in recognition. Gingival epithelial cells respond poorly to the FimA fimbriae, as they do not express CD14, a co-receptor for TLR2. This may limit the inflammatory responses to fimbriated, invasive *P. gingivalis*. Recognition of *P. gingivalis* proteases by protease activated receptors (PARs) can also lead to the production of cytokines and antimicrobial β-defensins.

Tissue destruction and bone loss

Bone remodeling is controlled to a large extent by the balance and relative activities of RANK (receptor activator of NF-κB ligand), RANK-L (RANK ligand) and osteoprotegerin (OPG). RANK-L is expressed by osteoblasts and T-cells, and can be up-regulated by IL-1, TNF-α and PGE2, whereupon it induces osteoclast production and activation. In periodontal pockets the presence of inflammatory cytokines such as IL-1 and activated T-cells can lead to bone resorption through the RANK/RANK-L system. The effects of RANK-L are antagonized by the decoy receptor OPG which acts as a soluble neutralizing receptor. IL-1 and IL-6 can also act on osteoclasts directly to stimulate bone resorption.

The formation of immune complexes in tissue may also contribute to tissue destruction. It is important to remember that a periodontal lesion is in essence a wound that fails to heal. Organisms such as *P. gingivalis* can degrade fibrinogen/fibrin and dysregulate tissue repair by cleavage and activation of matrix metalloproteinases.

Genetic factors associated with periodontal disease

As periodontal diseases are multifactorial, a large number of host genetic factors may be associated with susceptibility to the disease (see Table 26.2). Genetic defects leading to neutrophil dysfunction are strongly associated with disease as discussed above. In aggressive periodontitis there are often high levels of IgG2 antibodies to bacterial carbohydrates. IgG2 is unique in that production is dependent on Th1 cytokines. While Th1 responses are known to be under genetic control, the genes involved in this condition have yet to be fully characterized.

27 Relationship between periodontal diseases and systemic health

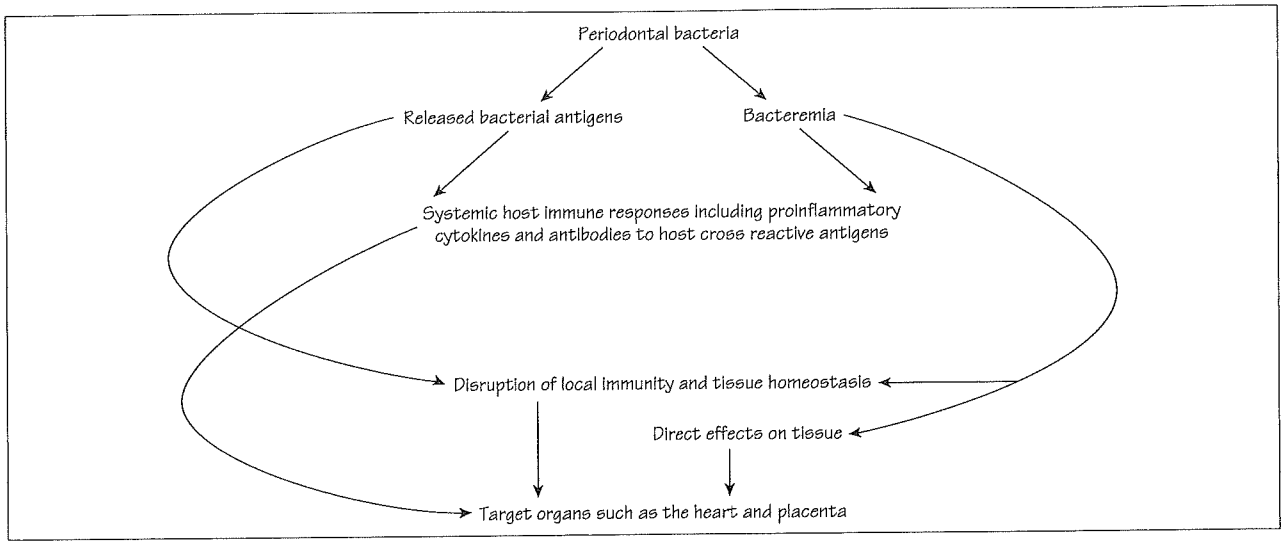

Figure 27.1 Schematic of the potential mechanisms linking periodontal status with systemic disease.

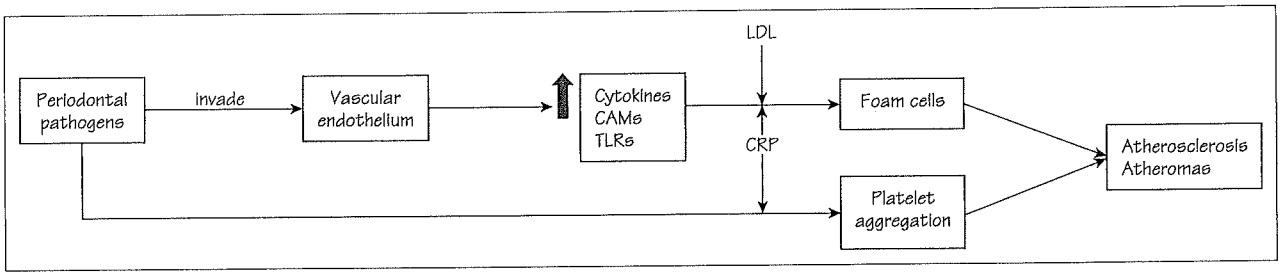

Figure 27.2 Possible connection between periodontal pathogens and cardiovascular disease.

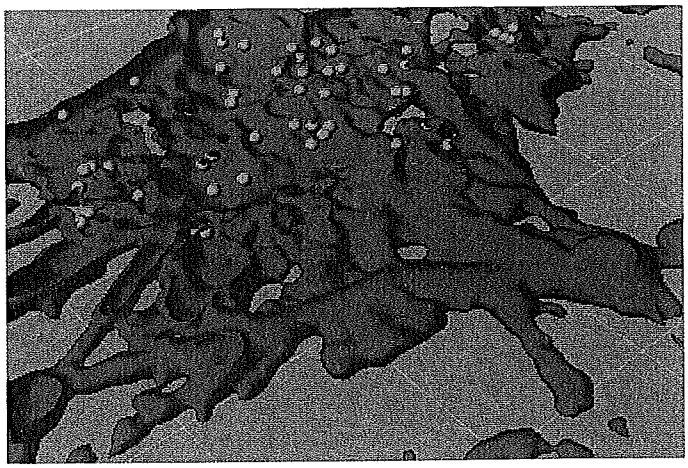

Figure 27.3 Cut away confocal microscopy image of a placental trophoblast cell (red) infected with *P. gingivalis* (green) and showing *P. gingivalis* is capable of internalizing within placental cells under experimental conditions.

While periodontal diseases are localized to the tissues supporting the teeth, evidence is emerging that periodontal infections and periodontal organisms are associated with serious systemic diseases such as cardiovascular disease, preterm delivery of low birth weight infants, pulmonary diseases, diabetes, osteoporosis and even Alzheimer's disease. Much of the evidence supporting these associations comes from epidemiological studies in which it is difficult to distinguish a causal relationship from an association resulting from a common predisposing factor. However, mechanistic bases for a direct causal link between periodontal diseases/organisms and systemic diseases is beginning to be established experimentally.

Pathogenic mechanisms – general principles

There are several broadly defined, and potentially interactive, mechanisms by which infections in the periodontal tissue could contribute to disease at remote sites (Figure 27.1). Periodontal bacteria and their antigens could initiate an inflammatory response in the periodontal tissues with systemic consequences. Moreover, bacteria and their products can gain access to the circulation during dental procedures such as scaling and root planing, or even after vigorous home care, especially in patients experiencing inflammation and tissue destruction such as occurs in periodontal disease. Once in the bloodstream, bacterial antigens could induce a systemic immune response. Periodontal bacteria in the blood could be carried to remote sites such as the placenta or heart tissues, either free in the circulation or within circulating cells such as monocytes or neutrophils, and initiate pathogenic processes at the remote sites. The ability of some periodontal bacteria, such as *P. gingivalis*, to resist both serum killing and oxygen-dependent killing within professional phagocytic cells would contribute to successful systemic dissemination. Finally, periodontal bacteria could initiate an autoimmune reaction, whereby bacterial antigens, such as heat shock proteins (HSPs) elicit a specific antibody that is cross-reactive with host molecules.

Cardiovascular disease (CVD)

Periodontal bacteria such as *P. gingivalis*, *T. forsythia*, *F. nucleatum*, *P. intermedia* and *A. actinomycetemcomitans* have been detected in carotid, coronary and aortic atheromatous plaques. It is important to note here, however, that detection was based on the presence of DNA, there has yet to be convincing evidence of live bacteria in atheromatous plaques. Nevertheless, *P. gingivalis* is capable of accelerating inflammatory plaque accumulation in the apolipoprotein E-knockout mouse model.

In vitro, *P. gingivalis* can invade and survive in endothelial cells, where it induces the secretion of proinflammatory cytokines and upregulates expression of cell adhesion molecules (CAMs) and TLRs. Invasion of the vascular endothelium *in vivo* would thus be predicted to result in a pro-inflammatory, pro-thrombotic environment that is characteristic of atherosclerosis. In the presence of low-density lipoprotein (LDL), *P. gingivalis* can also induce foam cell formation by macrophages, a hallmark of early atherogenesis. Platelet aggregation can be induced by *P. gingivalis*, which could precede thrombo-embolic events (Figure 27.2).

A characteristic of both periodontal disease and CVD is elevated serum C-reactive protein (CRP). CRP can interfere with endothelial nitric oxide (NO) availability and is associated with the formation of a platelet-rich thrombus following plaque rupture or erosion, and with foam cell formation. CRP could thus provide a link between periodontal disease and CVD without the requirement for bacterial damage to vascular endothelium.

Adverse pregnancy outcomes

Periodontal diseases and organisms have been associated with pre-eclampsia and with preterm delivery of low birth weight infants (PLBW). *P. gingivalis* DNA has been detected in the amniotic fluid of pregnant women with threatened preterm labor, and *F. nucleatum* has been cultured from the amniotic fluid of women in preterm labor. In animal models both *P. gingivalis* and *F. nucleatum* are capable of reaching the placental tissues and inducing fetal death and PLBW. *P. gingivalis* is also capable of invading placental trophoblast cells in culture (Figure 27.3).

Pregnancy is maintained through a balance of cytokines and chemokines, proteases and hormones. Microbial infection can disrupt this balance and lead to PLBW. In the mouse model, *F. nucleatum* induces fetal death through stimulation of TLR4-mediated placental inflammatory responses.

Pulmonary infections

Periodontal bacteria and biofilm-derived aggregates that are inherently resistant to phagocytosis can be shed into the saliva and then aspirated into the lower respiratory tract and the lungs. Periodontal organisms may cause infection directly or cause local tissue damage that would facilitate infection by more traditional respiratory tract pathogens. Cytokines produced in response to periodontal bacteria may also reach the lungs, where they can stimulate local inflammatory processes that facilitate subsequent bacterial colonization and tissue damage.

Diabetes

While diabetes is a risk factor for periodontitis, conversely periodontal infection can result in increased cytokine production along with activation of acute-phase protein synthesis, and consequent insulin resistance. For example, TNF-α negatively regulates insulin signaling and glucose uptake, and IL-6 impairs the glucose-stimulated release of insulin from isolated pancreatic beta cells. Both IL-1β and IL-6 are capable of antagonizing insulin action.

Osteoporosis

Bone loss is a feature shared between periodontal disease and osteoporosis. The mechanisms by which periodontal bacterial can cause alveolar bone loss (Chapter 26), for example by RANK-L activation, could contribute to skeletal loss of bone mass and bone density.

28 Implants and titanium

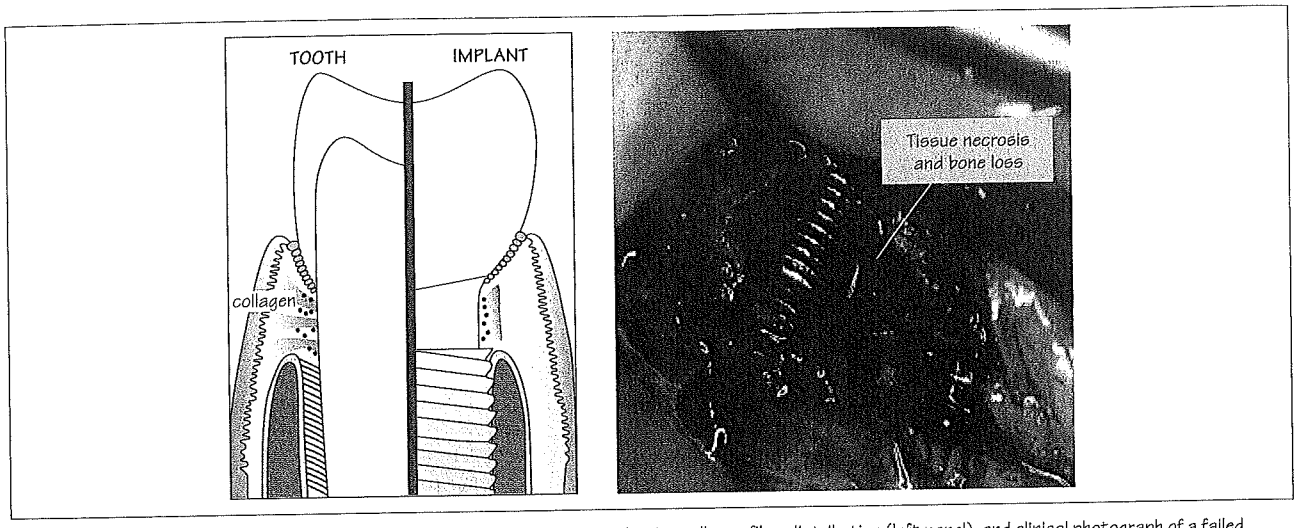

Figure 28.1 Diagrammatic vertical section comparison of tooth and implant showing collagen fiber distribution (left panel), and clinical photograph of a failed implant (right panel).

Table 28.1 Properties of titanium surfaces. Any titanium implant surface consists of an outer layer of titanium dioxide. The characteristics of this oxide layer may be divided into composition, topography and surface energy (wettability). All these affect biological reactions at the implant surface. Surface modifications may be used to improve bone bonding.

Surface topography	Implants come with roughened surfaces to improve bone bonding. Microsized topography can stimulate cell function and improve mechanical interlocking. Nanotopography is being developed to target specific cell functions for better control of cell behaviour
Composition	Calcium and phosphate (elements of hydroxyapatitie) may be incorporated into implant surfaces to match bone tissue (biomimetic). Crystallinity (the way Ti and O atoms are arranged) is also believed to affect implant performance
Wettabiliy	The wettability, or hydrophilicity, of an implant surface is very important for protein and cell adhesion. Some commercially available implants are kept in isotonic solution
Functionally loaded	Next generation implants may be more intelligent with biological molecules such as growth factors or specific proteins incorporated onto the surfaces (functionally loaded surface). The ability to slowly release such biological factors from an implant inserted into bone is also desirable

Table 28.2 Organisms most commonly isolated from peri-implantitis lesions

Microorganisms associated with peri-implantitis
Porphyromonas gingivalis
Prevotella intermedia
Fusobacterium species
Aggregatibacter actinomycetemcomitans
Staphylococcus aureus
Enterococcus faecalis
Candida albicans

Dental implants are becoming more common in the clinical setting. They are used in the repair of accidental damage to the jaw or dentition, or to replace teeth lost through decay or affected by trauma. In the most routine surgical procedure, a titanium (Ti) screw barrel is inserted into alveolar bone, usually into a vacant tooth socket. There is then a period of convalescence for the subject when the titanium implant is allowed to become stably incorporated into alveolar bone. This process is termed osseo-integration and is crucial to the success of the implant. A suitably shaped and colored artificial tooth is then screwed into the titanium barrel (see Figure 28.1). Because bone growth is stimulated by mechanical force or load, there has to be mechanical load applied to the implant over a period of time. Techniques being developed to speed up this process, by providing high loads at early stages, are risky. Implants should never be inserted into weakened alveolar bone or at inflamed sites. Teeth that have been lost as a result of periodontitis are not substitutable by implants because the implant will generally fail to osseo-integrate.

Osseo-integration

There are two important classes of cells involved in bone production and turnover. Osteoblasts are fibroblast-like cells that produce collagen and are responsible for bone cell development and mineralization. Osteoclasts are derived from cells in the bone marrow. They regulate bone production by removing the mineralized matrix, termed resorption, and remodeling bone structure. Titanium surfaces that allow bone cells (osteoblasts) to attach to them would be better integrated into the bone (Table 28.1). Growth factors and other compounds can improve the attachment or differentiation of osteoblasts growing in association with titanium. Currently, it is thought that oxidized titanium surfaces are more effective for cell attachment. Regular patterned arrays of only micrometer dimensions may be machined across the surface of titanium to promote osteoblast attachment and spreading.

Implant structure

There are differences in tissue structure of the mucosa surrounding the implant, compared with that surrounding the tooth root. In the latter (Figure 28.1 left panel) bundles of collagen fibers radiate out into the cementum of the root. With implants, collagen fiber bundles run parallel to the surface of the implant (Figure 28.1). Also, there are differences in composition of the connective tissue. The connective tissue of the mucosa around the implant has high collagen content. It is more similar to scar tissue and contains a much reduced blood supply. This contributes to a general decrease in immune function and to an increase in susceptibility to peri-implant infections.

Peri-implantitis

This condition refers to microbial infections that occur following implant surgery. Infection may be early after surgery, shown by redness, inflammation, discharge and rapid loss of bone. However, disease may take much longer to develop, resulting in alveolar bone resorption and rejection of the implant (Figure 28.1 right panel). The inflammation and inflammatory process goes deeper and faster around an implant compared to an adjacent natural tooth. Once this happens it is unlikely that a subsequent implant will be successful. The disease is very much like periodontal disease, but is acute and more destructive.

Bacteria associated with peri-implantitis

In disease conditions, the bacteria associated with the natural tooth or implant are very similar. They include *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium* and *A. actinomycetemcomitans*. However, there is often a much higher incidence of motile spirochetes at implant sites. Other microorganisms involved with infections at implant sites are varied. *Staphylococcus aureus*, enteric bacteria, such as *E. faecalis* and *Candida albicans* are commonly often found in peri-implant lesions (Table 28.2). By contrast, implants surrounded by healthy tissue demonstrate a microbiota associated with periodontal health. All peri-implant infections have a common feature: if they are not treated the infection leads to loss of the implant.

Asepsis and treatment

Dental implants are becoming increasingly important in prosthodontic rehabilitation. Bacterial infections, however, can induce bone loss and jeopardize clinical success. One area of concern is that the implants are properly sterile before insertion. Recently it has been demonstrated that infra-red CO_2 laser light is suitable for the decontamination of exposed implant surfaces. Another treatment regimen, photodynamic therapy (PDT), involves the use of a non-toxic dye (a photosensitizer) and low-intensity laser light. These combine to create singlet oxygen molecules that are lethal to certain bacteria. Laser treatment is best combined with surgical opening of the implant site for cleaning and disinfecting the local defect. In this way, photodynamic therapy can be used successfully to decontaminate the implant surface.

29 Endodontic infections

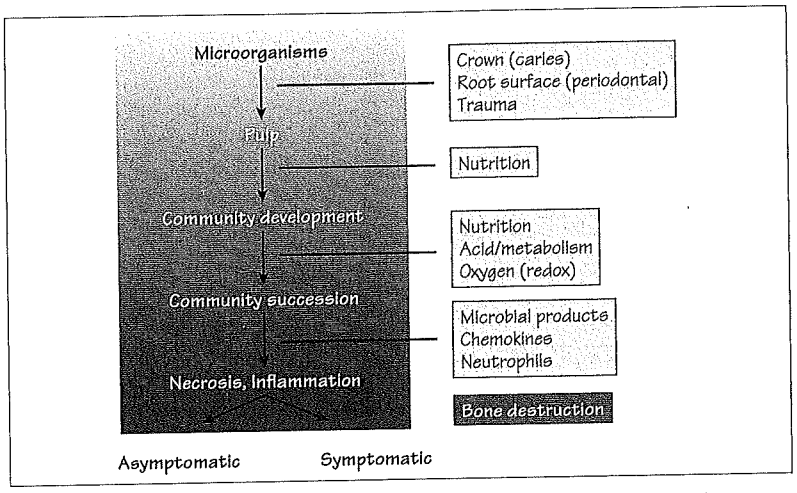

Figure 29.1 Course of endodontic infection following entry of microorganisms into the pulp.

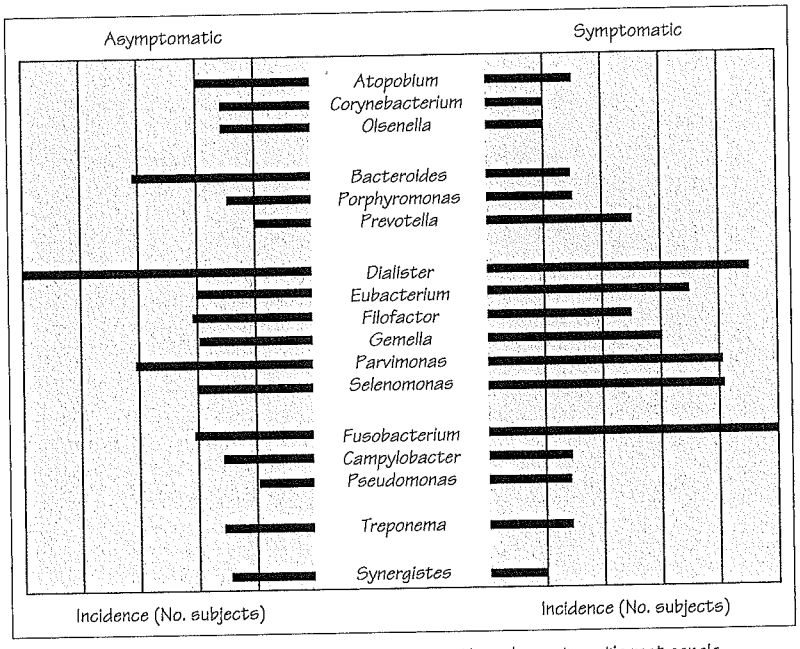

Figure 29.2 Microorganisms recovered from asymptomatic and symptomatic root canals.

Table 29.1 Cultivable microorganisms recovered from infected root canals.

Most prevalent cultivable taxa from infected root canals include:
Fusobacterium nucleatum
Porphyromonas gingivalis
Pseudoramibacter alactolyticus
Parvimonas micra (P. micros)
Streptococcus mitis
Streptococcus oralis/sanguinis/intermedius
Dominated by anaerobic bacteria
Most prevalent genus is *Streptococcus*

Table 29.2 Species diversity in infected root canals.

Phylotypes common to asymptomatic and symptomatic endodontic infections:
Dialister, Fusobacterium, Prevotella, Veillonella
Most common organisms detected by molecular studies independently of symptoms:
Porphyromonas endodontalis, Filofactor alocis, Porphyromonas gingivalis, Tannerella forsythia, Pseudoramibacter alactolyticus
Archaea (*Methanobrevibacter*) convert H_2 to methane
Root canal samples from asymptomatic infections show higher diversity of species

Endodontic infections refer to those that occur within the tooth pulp, root canal system or at the root apex (Figure 29.1). Normally the pulp and root canal system are sterile. However, bacteria may enter through cracks around restorations, areas of exposed dentin and possibly microfracture, or through trauma to the tooth. There is also a theory that microbes present transiently in the circulatory system could become lodged within the apical region of the root and cause abscess formation. One of the major areas of debate is how exactly complex populations of bacteria form inside the root canal system. However, there is good evidence that oral bacteria may penetrate the dentinal tubules.

Dentinal tubules

Dentinal tubules are present within and across dentin. They are produced during dentin formation by odontoblast migration. The tubules are wider at the pulpal side (approx. 2.5 μm) and contain more collagen fibers. The tubules are narrower at the dentino-enamel junction side (approx. 0.9 μm) and more calcified. Dentinal fluid (containing albumin, transferrin, tenascin and protoglycans) is present within the tubules, giving dentin a permeability which increases the sensation of pain. Bacteria may penetrate these tubules if dentin is exposed, or invade the tubules from the pulpal side. Bacteria present within tubules are hard to eliminate. They are well protected from defense molecules and from antiseptics used in endodontic surgery. Bacterial infection affects the hydrostatic pressure within tubules, increasing the sensitivity to hot or cold liquids or food.

Bacterial invasion of dentin

The populations of bacteria present in long-term root canal and pulpal infections (pulpitis) are complex. In laboratory experiments, dentin is most quickly infected by bacteria that are not necessarily main components of the root canal microbiota. For example, *S. mutans* and *S. gordonii* readily infect dentinal tubules, and can penetrate to depths of 0.2 mm or more over several days. *P. gingivalis* cannot penetrate dentinal tubules in pure culture, but can invade in combination with streptococci to which it attaches. Thus, direct microbial interactions may be important in generating root canal microbiota.

Microbiota of endodontic infections

Studies of the endodontic bacteria have relied heavily on anaerobic cultivation techniques to identify the components. However, molecular approaches have considerably improved the analyses, showing that some initial interpretations were incorrect. For example, in many cases *Entercococcus faecalis* was isolated as a component of early root canal infections. It is clear now that this organism, whilst found in some cases, is not the major pathogen that it was believed to be. Somewhere between 50 and 60 taxa are found associated with endodontic infections. The most prevalent cultivable taxa from root canals are *F. nucleatum*, *P. gingivalis*, *Pseudoramibacter alactolyticus*, *Parvimonas micra (Peptostreptococcus micros)*, *S. mitis*, *S. intermedius*, other streptococci and *Candida*. The microbiota is dominated by anaerobic bacteria, but the most prevalent genus is *Streptococcus* (Table 29.1). Molecular methods reveal that there are many bacteria present that are uncultivable, including *Treponema*, *Filofactor alocis*, *Dialister pneumosintes*, *Dialister invisus*, *Olsenella uli* and *Olsenella profusa*. The most common organisms detected by molecular studies, independently of symptoms, are *Porphyromonas endodontalis*, *Filofactor alocis*, *P. gingivalis*, *T. forsythia*, and *P. alactolyticus*. Archaea are also present, e.g. *Methanobrevibacter* that converts H_2 to methane (Table 29.2). About 50% of the bacteria will not grow on artificial media. This may be due to lack of essential nutrients, medium toxicity, or dependence upon another species. Primary infections have been found to contain 10–30 species of bacteria. However, persistent infections contain fewer species. No single species of bacterium is recognized as a pathogen.

Symptomatic versus asymptomatic

There has been considerable debate about whether or not different species of bacteria are associated with symptomatic (pain) or asymptomatic conditions. Phylotypes common to both asymptomatic and symptomatic endodontic infections are *Dialister*, *Fusobacterium*, *Prevotella* and *Veillonella*. There is, however, some tentative data suggesting that the incidence of *Fusobacterium* and *Eubacterium* is higher in symptomatic conditions (Figure 29.2).

Treatment

Patients with acute pain are often given antibiotics, such as amoxicillin and metronidazole. However, the bacteria are so protected within the pulpal region that antibiotics are often of no benefit. Root canals are cleared of pulp and debris by the clinician, and characteristically irrigated with sodium hypochlorite, and a calcium hydroxide paste applied. Both these regimens are anti-bacterial, although some organisms, e.g. *E. faecalis* are more resistant. Because sodium hypochlorite also damages host tissues, other irrigants or sterilants have been promoted. One of these is ozone, which is bactericidal, but it is rapidly inactivated by organic matter.

30 Host factors in endodontic infections

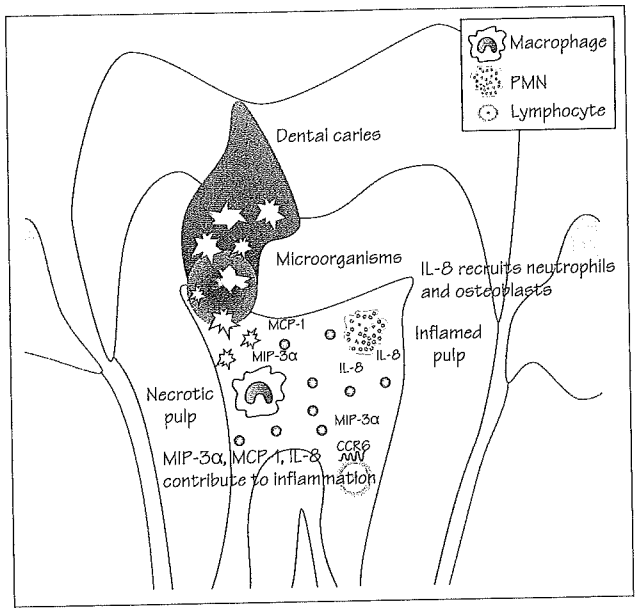

Figure 30.1 Generation of host immune responses to microorganisms within the pulp. Reproduced with permission from Silva TA *et al.* Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. *Journal of Dental Research* (2007); 86: 306–319.

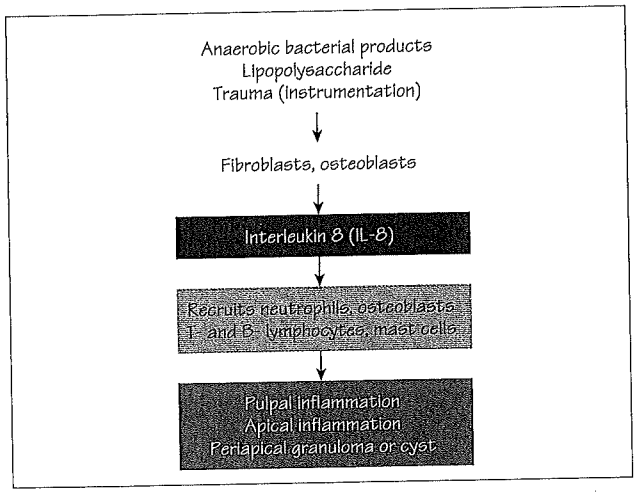

Figure 30.2 Key role of interleukin 8 in endodontic infections.

Table 30.1 Cytokines in apical periodontitis.

Roles of individual cytokines not known
Elevated IL-8/CXCL8, MIP-3a/CCL20, MCP-1/CCL-2 characteristic of inflamed pulp
MIP-3a/CCL20 produced by macrophages
IL-8 activity in cyst epithelial lining
Balance of cytokines, chemokines regulate migration, proliferation and matrix synthesis
Evaluation of lesion progression, e.g. active or healing

Clinical symptoms of endodontic infections result from inflammation of the pulp in response to microbial challenge. In these respects, endodontic infections are very much like periodontal infections. Pulpal infection can progress to the periapical region and generate alveolar bone destruction and chronic apical periodontitis. The immunology and host innate responses to microbial challenge are just beginning to be understood. Essentially, chemokines are generated in response to bacterial infection of pulp. These contribute to inflammation and recruit ostoeblasts and neutrophils. The pulpal inflammation may then progress to the apical region (Figure 30.1).

Pulpal infections

The inflammatory and immune responses that are initiated by bacteria are generated in order to protect the host against infection. The persistence of a local chronic response alters the protective role of inflammatory cells and produces deleterious effects. Development of periodontal disease is associated with progression of inflammatory cell infiltrate into the deeper periodontal tissues. Dental pulp is normally protected from the bacteria of the oral cavity by the presence of enamel and dentin. Exposure of dental pulp to bacteria as a result of dental caries, fractures, or operative procedures triggers a pulpal inflammatory response. Severe pulpitis, which occurs often as a result of dental caries, is characterized by the presence of a major inflammatory infiltrate. However, little is understood about how these cells are recruited into dental pulp lesions. Pulp cells are able to respond to bacteria and toxins through chemokine production. A high concentration of interleukin 8 (IL-8), a major chemo-attractant of PMNs, has been detected in pulps that have been diagnosed with irreversible pulpitis. Normal pulps show only weak, or no, IL-8.

Periapical abscesses

In inflamed pulp the chemokines MIP-3a, IL-8 and MCP-1 contribute to inflammatory cell infiltration (Table 30.1). Progression of pulpal inflammation to the periapical region leads to adaptive and innate immune responses. The outcome is periapical alveolar bone destruction and lesion formation. Combinations of bacteria appear to be more effective inducers of bone resorption than single species. Chronic apical periodontitis is referred to as periapical granuloma and can evolve to produce a periapical cyst. The main components of these lesions are PMNs, macrophages, T and B lymphocytes, mast cells, osteoclasts and osteoblasts. Chemokines are key elements in the formation of granulomas, and their production is elicited by bacteria. The predominantly anaerobic microbiota of the root canals (*P. gingivalis*, *P. endodontalis*, *Prevotella intermedia*, etc.) are all able to induce the production of IL-8 by pulp fibroblasts, and MIP-1α and MIP-1β by neutrophils. A positive association between IL-8 levels and painful symptoms has been reported. Chemokine production in periapical lesions may also be stimulated by trauma, injury from instrumentation or irritation from chemicals used in endodontics (Figure 30.2). Often, chemical and mechanical preparation of the root canal, in conjunction with filling of the root canal system, leads to elimination of an infection and healing of the periapical tissues. In some instances, however, apical periodontitis does not respond to treatment. It is thought that this is due to the anatomical complexity of root canals, which makes the elimination of microorganisms difficult. Host factors must also function suitably to effect repair. Thus, migration of lymphocytes, neutrophils and mononuclear cells is essential for the periapical tissue response.

Repair and angiogenesis

Chemokines are considered to be important for the recruitment of subpopulations of leukocytes. Cytokines can also help repair tissue and promote angiogenesis. Expression of MIP-1α has been linked to enhanced macrophage influx, angiogenic activity and collagen production. The higher expression of chemokines, e.g. MCP-1, CCR3, CCR5 and CXCR1 in cysts compared with granulomas may be significant in the development of granulomas to cysts. Chemokines might be useful for evaluating the progression of periapical lesions. It could be determined whether the lesions are active or healing by sampling through the root canal before obturation. This knowledge may provide new means for controlling apical periodontitis.

Resistant bacteria

Survival of bacteria within periapical tissues is probably the reason for endodontic treatment failures. The periapical microbiota in these instances is usually dominated by Gram-positive organisms and is different from the microbiota that appears to respond to treatment. Resistant organisms include *Streptococcus*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Enterococcus* and *Candida*. The organisms become adapted to live in the environment and may be surrounded by matrix as in a biofilm. The microorgansims responsible for long-term infections may not necessarily be conventional oral bacteria. For example, *Psuedomonas aeruginosa* has been found frequently to be an associated agent. Often, antibiotics such as metronidazole and carbenicillin, which may be effective in periodontal control, are ineffective in eliminating periapical infections.