PERIODONTOPATHOGENS

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INTRODUCTION

Periodontal diseases are infections that are caused by microorganisms that colonize the tooth surface at or below the gingival margin. It is estimated that about 500 different species are capable of colonizing the mouth and any individual may typically harbor 150 or more different species. Counts in sub gingival sites range from about 10 healthy, shallow sulci to > 10 in deep periodontal pockets. Numbers in supra gingival plaque can exceed 10 on a single tooth surface. Thus, while hundreds of millions or even billions of bacteria continually colonize die tooth at or below the gingival margin throughout life, most periodontal sites in most individuals are not exhibiting new loss of the supporting structures of the teeth at any given time. This recognition is critical. Occasionally, a subset of bacterial species is either introduced, overgrow or exhibit new properties that lead to the destruction of the periodontium. The resulting stressed equilibrium is usually spontaneously corrected, or corrected by therapy.

SIMILARITIES OF PERIODONTAL DISEASES TO OTHER INFECTIOUS DISEASES

Our concepts of infectious diseases often appear to be influenced by our experiences with acute infections, particularly upper respiratory infections. In acute in-fections, an agent is acquired by exposure to an individual harboring mat agent or from the environment. The agent establishes within tissues or on mucous membranes or skin. Within a short time, signs or symptoms of a disease appear at the site of introduction or elsewhere in the individual. A "battle" occurs between the parasite and the host resulting in increasingly obvious clinical signs and symptoms. This host-bacterial interaction is often resolved within a short time, usually, but not always, in favor of the host. Thus, daily experience suggests that colonization by a pathogen is rapidly followed by expression of disease. While certain infections follow this pattern, more commonly, colonization by a pathogenic species does not lead to overt disease, at least immediately. In a similar fashion individuals may be colonized continuously by periodontal pathogens at or below the gingival margin and yet not show evidence of ongoing or previous periodontal destruction. Many of the organisms that colonize such

Sites are members of species thought to be periodontal pathogens. In spite of their presence, periodontal tissue damage does not lake place. This is not an anomaly. This phenomenon is consistent with other infectious diseases in which it may be observed that a pathogen is necessary but not sufficient for a disease to occur.

Infectious diseases in a given organ system are caused by one or more of a relatively finite set of pathogens. Further, different species have different tissue specificities and cause diseases in different sites in the body. Periodontal diseases appear to be caused by a relatively finite group of periodontal pathogens acting alone or in combination. Such species include Actinobacillus actinomycetemcomitans, Bactericides forsythias, and Campylobacter rectus. Eupatorium no datum, Fusobacterium nucleatum. Peptostreptococcus micros. Porphyromonas gingivalis, Prevotella intermedia. Prevotella nigrescens, Streptococcus intermedius and Treponema sp. (Haffajee & Socransky 1994).

There are a number of other common themes observed in different infectious diseases, particularly those that affect mucous membranes, such as the need to attach to one or more surfaces, the need to "sense" the environment and turn on or off various virulence factors and the need to overcome or evade host defense mechanisms. Infectious agents have evolved a set of common strategies to perform these tasks and the host has developed a series of responses to combat these infections. Thus, periodontal diseases are infectious diseases that have many properties that are similar to bacterial infections in other parts of the body and to a large extent can be combated in similar fashions.

UNIQUE FEATURES OF PERIODONTAL INFECTIONS

Although periodontal diseases have certain features in common with other infectious diseases, there are a number of features of these diseases that are quite different. In certain ways periodontal diseases may be among the most unusual infections of the human. The major reason for this uniqueness is the unusual anatomic feature that a mineralized structure, the tooth, passes through the integument, so that part of it is exposed to the external environment while part is within the connective tissues. The tooth provides a surface for the colonization of a diverse array of bacterial species. Bacteria may attach to the tooth itself, to the epithelial surfaces of the gingiva or periodontal pocket, to underlying connective tissues, if exposed, and to other

bacteria which are attached to these surfaces. In contrast to the outer surface of most parts of the body, the outer layers of the tooth do not "shed" and thus microbial colonization (accumulation) is facilitated. Thus, a situation is set up in which microorganisms colonize a relatively stable surface, the tooth, and are continually held in immediate proximity to the soft tissues of the periodontium. This poses a potential threat to those tissues and indeed to the host itself.

The organisms that cause periodontal diseases reside in biofilms that exist on tooth or epithelial surfaces. The biofilm provides a protective environment for the colonizing organisms and fosters metabolic properties that would not be possible if the species existed in a free-living (plank tonic) state. The presence of a tooth increases the complexity of the host parasite relationship in a number of ways. The bacteria colonizing the tooth are by and large outside the body where they are less able to be controlled by the potent mechanisms which operate within the tissues. The environment within a plaque may be conducive for microbial survival, but it is unlikely to be a particularly effective environment for the host to seek out and destroy microorganisms. Factors such as hydrogen ion concentration (pH),oxidation reduction potential (Eh),and proteolytic enzymes, can affect the performance of host defense mechanisms. In addition, the tooth provides "sanctuaries" in which microorganisms can hide, persist at low levels during treatment and then re-emerge to cause further problems. Bacteria in dentinal tubules, flaws in the tooth, or areas that were demineralized by bacteria are not easily approached by the much larger host cells. In a similar fashion, non-cellular host factors must face diffusion barriers, lytic enzymes and absorption by the mineral structure of the tooth. Mechanical debridement other than vigorous removal of tooth material cannot reach organisms within the tooth. Chemotherapeutic agents will also have difficulty in reaching the organisms.

Taken together, the infections which affect the tooth and its supporting structures present a formidable problem for both the host and the therapist. The unique anatomical features of this "organ system" must be borne in mind as we attempt to unravel the etiology and pathogenesis of periodontal diseases and plan treatment or prevention strategies for their control.

HISTORICAL PERSPECTIVE

The search started in the "*golden age of microbiology*" (approx. 1880-1920), when the etiologic agents of many medically important infections were determined. It is not surprising that parallel investigations of the etiology of periodontal diseases were initiated in this era. However, these investigations were not as successful as some of the investigations of extra oral infectious discuses.

THE EARLY SEARCH

Investigators in the period from 1880 to 1930 suggested four distinct groups of microorganisms as possible etiologic agents: *amoeba, spirochetes, fusiforms and streptococci.* The basis of this determination was primarily the seeming association of these organisms with periodontal lesions. The identification of a suspected pathogen was heavily influenced by the nature of the techniques available. The major techniques at that time were wet mount or stained .smear microscopy and limited cultural. The different techniques suggested different etiologic agents.

Amoeba

Certain groups of investigators used stained smears to seek amoeba in bacteria! Plaque. They found higher proportions of amoebae in lesions of destructive periodontal diseases than in samples taken from sites from healthy mouths or mouths with gingivitis. Local therapies for this organism included the use of dyes or other antiseptic agents to decrease the numbers of amoebae in the oral cavity. Other approaches employed agents such as the emetic, emitin, administered systemically or locally The role of amoeba in periodontal disease was questioned by some author because amoeba were found in sites with minimal or no disease and could not be detected in many sites destructive disease, and because of the failure of emitin to ameliorate the symptoms of the disease.

Spirochetes

Other investigators used wet mount preparations, specific stains for spirochetes when

they examine dental plaque. They reported higher proportions spirochetes and other motile forms in lesions of destructive disease when compared with control sites in the same or other individuals. This finding led to suggestion that spirochetes may be etiologic agents for destructive periodontal disease. Therapies were posed that sought to control disease by the elimination or suppression of these microorganisms including systemic administration of Neosalvarsan (compound 606), the anti-spirochetal agent used to treat syphilis coupled with the use of sub gingival scaling to control destructive periodontal disease. Other investigators employed bismuth compounds to treat oral spirochetal infections. Many investigators claimed success controlling advanced destructive periodontal disease by combining local and systemic therapy. Others questioned the relationship of spirochetes to periodontal diseases.

Fusiforms

The third group of organisms which were frequently suggested to be etiologic agents of destructive periodontal diseases, including Vincent's infection, were the spindle-shaped fusiforms. These organisms were originally recognized on the basis of their frequently appearance in microscopic examination of sub gingival plaque samples. The organisms were first related to periodontal disease by Plaut (1894). Vincent (18 99) distinguished certain pseudomembraneous lesion the oral cavity and throat from diphtheria and recognized the important role of fusiforms and spirochetes in this disease. In honor of this investigator the infection became known as Vincent's infection. The important role of spirochetes and fusiforms in Vincent's infection was widely recognized in the succeed two decades.

Streptococci

The fourth group of microorganisms which were proposed as etiologic agents of periodontal diseases in this era were the streptococci. These microorganisms were proposed on the basis of cultural examination of samples of plaque from sub gingival sites of periodontal disease. The selection of the streptococci may have been predicated upon the fact that these were the only species that could be consistently isolated from periodontitis lesions using the cultural techniques of that era. Among this group, the streptococci would have been most prominent. Since there were no methods

Available at that time for the specific control of streptococci, workers turned to nonspecific agents such as intramuscular injection of mercury or to the use of, vaccines for the control of periodontal diseases.

Vaccines

For the first three decades of the twentieth century, vaccines were commonly employed by physicians and dentists in attempts to control bacterial infections. Three types of vaccines were employed for the control .of periodontal diseases. These included vaccines prepared from pure cultures of streptococci, and other oral organisms, autogenous vaccines and stock vaccines such as VanCott's vaccine, Goldenberg's vaccine or Inava Endocorps vaccine. These vaccines were administered systemically or locally in the periodontal tissues.

Autogenous vaccines were prepared from the dental plaque of patients with destructive periodontal ceases. Plaque samples were removed from the diseased site, "sterilized" by heat and/or by immersion of iodine or formalin solutions, and then re-injected into same patient, either in the local periodontal lesion or systemically. Proponents of all three techniques claimed great efficacy for the vaccination methods employed, while others using the same techniques were more skeptical

Other forms of therapy directed against oral microorganisms

Ultraviolet light was widely used to attempt to control the oral microbiota and to improve the well-being of the local tissue.

Electrochemical techniques, caustic agents such as phenol, sulfuric, trichloracetic or chromic acids, nascent copper and castor oil soap (sodium ricolineate), and even radium was used to combat root canal infections.

One technique which appears to have been commonly employed was the use of sodium sulfide to "dissolve" the epithelial lining of the pocket and permit reattachment.

Invasion - the early years

One of the more interesting phenomena of research is the fact that research workers keep rediscovering the same phenomena in a cyclical fashion. Invasion of the periodontal tissues by bacteria was thought to be important in the pathogenesis of periodontal diseases in the early 1900s, was forgotten and then re-discovered.

Beckwith et al. (1925) used stains specific for bacteria to study biopsy specimens from prisoners at San Quentm who had periodontitis. They regularly observed bacteria both within the epithelium and in the underlying tissues. Bacteria in the epithelium were usually streptococci or "diptheroids". Gram-negative rods were observed in the connective tissue. They noted the rare occurrence of spiral forms in the tissues, although they were routinely detected in the plaque overlying the tissues. Invasion of spirochetes deep into the lesions of Vincent's infection was clearly documented. It was thought that the spirochetes moved into the connective tissues first and were followed by fusiform-shaped species.

The decline of interest in microorganisms

The initial enthusiasm for the hunt for the etiologic agents of destructive periodontal diseases slowly subsided and by the mid-1930s there were virtually no workers involved in this quest. This state was eloquently described by Belding & Belding (1936) in the aptly titled "Bacteria - Dental Orphans". During the period from the mid-1920s to the early 1960s, the attitude toward the etiology of periodontal disease changed. In the first two decades of this period it was thought that periodontal disease was due to some constitutional defect on the part of the patient, to trauma from occlusion, to disuse atrophy or to some combination of these factors. Bacteria were thought to be merely secondary invaders in this process or at most, contributors to the inflammation observed in periodontal destruction.

Non-specific plaque hypothesis

Treatment of patients based on the notion of constitutional defects or trauma from occlusion was not effective in controlling periodontal diseases. Clinicians recognized that plaque control was essential in the satisfactory treatment of periodontal patients. During the late 1950s, a group of clinicians, sometimes referred to as "plaque evangelists", heavily emphasized the need for plaque control in the prevention and

treatment of periodontal diseases. Thus, once again bacteria were thought to play a role in the etiology of destructive periodontal disease, but as non-specific causative agents. According to this "non-specific plaque" hypothesis, any accumulations of microorganisms at or below the gingival margin would produce irritants leading to inflammation. The inflammation in turn was responsible for the periodontal tissue destruction. The specific species of microorganisms that accumulated on the teeth was not considered to be particularly significant providing that their numbers were sufficiently large to trigger a destructive process.

Mixed anaerobic infections

Beginning in the late 1920s, a series of oral and medical microbiologists believed that periodontal disease was the result of "mixed infections". This hypothesis had been considered since the late 1800s when microscopic observations by Vincent in France suggested that certain forms of periodontal disease, particularly acute necrotizing ulcerative gingivitis (ANUG), were due to a complex of microorganisms dominated by fusiforms and spirochetes. These infections were known as fuso-spirochetal infections. In the early 1930s, investigators found that mixtures of microorganisms taken from lung infections or sub gingival plaque would induce lesions when subcutaneously injected into various experimental animals. A combination of a fusiform, a spirochete, an anaerobic vibrio and an alpha hemolytic streptococcus could cause transmissible infections in the guinea pig. Later investigators failed to reproduce their results either with the above combination of microorganisms or with many other combinations they tested. They did demonstrate, however, that mixed infections were due to bacteria {rather than a virus).

Macdonald and co-workers (1956) were later able to produce transmissible mixed infections in the guinea pig groin using combinations of pure cultures. The critical mixture of four organisms included a *Bacteroides melaninogenicus* strain, a Gram-positive anaerobic rod and two other Gram-negative anaerobic rods. This combination of organisms was completely different from those used by earlier investigators to cause transmissible infections. These results led to the concept that mixed infections might be "bacteriologically non-specific but biochemically specific". In other words, any

combination of microorganisms capable of producing an array of destructive metabolites could lead to transmissible infections in animals and, by extension, to destructive periodontal infections in humans. Later experiments suggested that members of the B. *melaninogenicus* group were the key species in these infections.

RETURN TO SPECIFICITY IN MICROBIAL ETIOLOGY OF PERIODONTAL DISEASES

In the 1960s, interest in the specific microbial etiology of periodontal disease was rekindled by two groups of experiments. The first demonstrated that periodontal disease could be transmitted in the hamster from animals with periodontal disease to animals without periodontal disease by caging them together. Swabs of plaque or feces from diseased animals were effective in transmitting the disease to animals free of disease. It was demonstrated that a pure culture of an organism that later became known as *Actinomyces viscosus was* capable of causing destructive periodontal disease in animals free of disease. Other species isolated from the plaques of hamsters with periodontal disease did not have this capability.

At about the same time, it was demonstrated that spirochetes with a unique ultra structural morphology could be detected in practically pure cultures in the connective tissue underlying lesions of ANUG and within the adjacent epithelium. Control tissue taken from healthy individuals and individuals with other forms of disease did not exhibit a similar tissue invasion. To date, the spirochete associated with ANUG has not been cultivated.

Such findings suggested that there might be more specificity to the microbial etiology of periodontal disease than had been accepted for the previous four decades. However, the emphasis in the 1960s was on mechanical control of plaque accumulation. This approach was consistent with the prevailing concept at periodontal disease was due to a non-specific accumulation of bacteria on tooth surfaces. This concept is very much. in evidence today and still serves as the basis of preventive techniques in most dental practices. It is also clear that non-specific plaque control is not able to effectively prevent all forms of periodontal disease.

The transmissibility studies stimulated a new concept of periodontal diseases. The

organisms which were responsible for the periodontal destruction observed in the hamster clearly differed from other organisms by their ability to form large amounts of. bacterial plaque both in the hamster and in *vitro* test systems. A concept emerged that microorganisms that were capable of forming large amounts of plaque *in vivo* and *in vitro* should be considered as prime suspects in the etiology of periodontal diseases. Human isolates of *Actinomyces* species were shown to have this ability *in vitro* and led to plaque formation and; periodontal destruction in animal model systems. These findings reinforced the notion that organisms; that formed abundant plaque were responsible for destructive periodontal disease. Unfortunately, later research findings revealed major discrepancies in this hypothesis.

CHANGING CONCEPTS OF THE MICROBIAL ETIOLOGY, OF PERIODONTAL DISEASES

By the end of the 1960s it was generally accepted that | dental plaque was in some way associated with human periodontal disease. It was believed that the presence of bacterial plaque initiated a series of as yet undefined events that led to the destruction of the periodontium. The composition of plaque was thought to be relatively similar from patient to patient and from site to site within patients. Variability was recognized, but the true extent of differences in bacterial composition was not appreciated. It was thought that the major event triggering destructive periodontal disease was an increase in mass of bacterial plaque, possibly accompanied by a diminution of host resistance. Indeed, in the mid 1960s the classic studies of Loe, Theiiade and co-workers (Loe et al. 1965, 1967, Theiiade et al. 1966) convincingly demonstrated that plaque accumulation directly preceded and initiated gingivitis. Many investigators believed that gingivitis was harmful and led to the eventual destruction of the periodontal tissues, probably by host-mediated events. Yet. certain discrepancies continued to baffle clinicians and research workers alike.

Explanation may have been that there were inconsistencies in the host response, or disease required the superimposition of local factors such as trauma from occlusion, overhanging fillings, etc. Other explanations can be derived from more recent studies of the microbiology of periodontal diseases. The recognition of differences in the composition of bacterial plaque from subject to subject and site to site within subjects

led to a series of investigations. Some studies attempted to determine whether specific microorganisms were found in lesion sites as compared to healthy sites. Other studies sought differences in the microorganisms in subgingival plaque samples taken from subjects with clinically different forms of periodontal disease. Newman and co-workers (1976, 1977) and Slots (1976) demonstrated that the microbial composition of sub gingival plaque taken from diseased sites differed substantially from the samples taken from healthy sites in subjects with localized juvenile periodontitis (LJP). Tanner et al. (1979) and Slots (1977) demonstrated that the microbiota recovered from lesion sites from subjects with adult periodontitis differed from the microbiota from healthy sites in the same subjects and also from lesion sites in LJP subjects. These studies along with the demonstration that subjects with LJP could be treated successfully with local debridement and systemic antibiotics

CRITERIA FOR IDENTIFICATION OF PERIODONTAL PATHOGENS

In the 1870s, Robert Koch developed the classic criteria by which a microorganism can be judged to be a causative agent in human infections. These criteria, known as *Koch's postulates,* stipulate that the causative agent must:

1. Be routinely isolated from diseased individuals
2. Be grown in pure culture in the laboratory
3. Produce a similar disease when inoculated into susceptible laboratory animals
4. Be recovered from lesions in a diseased laboratory animal

Streptococcus mutans, for example, has been shown to fulfill Koch's postulates as an etiologic agent of dental caries. However, difficulties exist in the application of these criteria to other types of diseases, and the applicability of Koch's postulates has been increasingly challenged in recent years. In the case of periodontitis. three primary problems are the inability to culture all the organisms that have been associated with disease (e.g., many of the oral spirochetes), the difficulties inherent in defining and culturing sites of active disease, and the lack of a good animal model system for the

study of periodontitis.

Sigmund Socransky, a researcher at the Forsyth Dental Center in Boston, proposed criteria by which periodontal microorganisms may be judged to be potential pathogens. According to these criteria, a potential pathogen must:

1. Be associated with disease, as evident by increases in the number of organisms at diseased sites
2. Be eliminated or decreased in sites that demonstrate clinical resolution of disease with treatment
3. Demonstrate a host response, in the form of an alteration in the host cellular or humoral immune response
4. Be capable of causing disease in experimental animal models
5. Demonstrate virulence factors responsible for enabling the microorganism to cause destruction of the periodontal tissues

MICROBIAL CLASSIFICATION

Classification is the arrangement of organisms into groups (taxa) on the basis of their similarities and differences. In contrast, identification is the process of determining. that a new isolate belongs to a particular taxon; the aim of classification is to define these taxa at the genus or species level. Traditionally, a hierarchical system has existed for the naming of bacteria so that groups of closely related organisms form a species, and related species are placed in a genus etc. species are designated by Latin or Latinized binomials (e.g. Streptococcus mutans; the genus is Streptococcus and the species is mutans. If an isolate belong to an existing taxon, then a new species can be proposed. The naming of bacteria to reflect this classification (nomenclature) is regulated by an international committee. Once an organism has been placed in a species, it may be possible to sub-type individual strains: this can be valuable in epidemiological studies investigating transmission of strains between individuals. The classification nomenclature and identification of microorganisms is referred to as taxonomy; although sometimes, the terms classification and taxonomy are used interchangeably. Early classification schemes relied heavily on morphological and simple physiological criteria, e.g. the shape of the cell, and the pattern of fermentation of simple sugars. In effect," these approaches analyzed only a fraction of the genetic material of the cell

(genome). In contrast, contemporary classification schemes arc based more on chemical analyses of cells (chemotaxonomy) and on measuring the genetic relatedness among strains. Chemotaxonomy compares the molecular composition of major components of whole cells {e.g. membrane lipids, peptidoglycan "structure, whole cell protein profiles, fermentation products) and hence a greater number of gene products. Likewise, the antigenic profile of cell surfaces can be compared using serological techniques (serology), using specific antibodies (polyclonal or monoclonal).

As the properties of an organism are dictated by what is coded in its genome, the ultimate comparison is to determine the similarity in DNA base composition. Initially, the mole percentage of guanine (G) plus cytosine (C) in the total DNA is determined. Organisms with markedly different *G+C* contents are .unrelated, while organisms that have similar G+C values are closely related, although similarity in gross DNA composition is not unequivocal proof of close relatedness because the base pairs could be organized in a different sequence. In such a situation, genotypic similarity can be confirmed by determining the degree of homology between the DNA from two strains, i.e. the abilities of heat-denatured, single strands of DNA from different strains to Renewal with each other, or to a reference strain, during slow cooling (DNA-DNA hybridization). High levels of homology reflect an overall similarity, in the nucleotide sequences from the DNA of the two strains, and hence confirms their close taxonomic relationship. The genetic relatedness of micro-organisms can also be determined by comparing 16S ribosomal RNA (16S rRNA') sequences. This approach has clarified the classification of many heterogeneous, groups. These gene sequences have also been exploited in the design of species-specific probes or primers for identification purposes the advent of molecular approaches to classification has allowed bacteria to be grouped for the first time according to their 'natural' relationships, thereby showing their evolutionary relationships as phylogenetic trees.)

A consequence of classification is the generation of internationally approved species. A species represents a collection of strains that share many features in common, and which differ considerably from other strains. Once a species has been recognized, then a type strain is nominated that has properties representative of the species. Type strains arc held in national collections, such as the American Type Culture Collection

(ATCC), and the National Collection of Type Cultures (NCTC) which is located in the United Kingdom A species may be divided into sub-species if minor but consistent phenotypic variations can be recognized. Likewise, groups of strains within a species can sometimes be distinguished by a special characteristic, and are termed biovars or **biotypes,** while strains with a distinctive antigenic composition are described as serovars or serotypes, and can be recognized using appropriate anti-bodies.

DIFFICULTIES IN DEFINING THE MICROBIAL PATHOGENS IN DESTRUCTIVE PERIODONTAL DISEASES

The search for the etiological agents of destructive periodontal diseases has been in progress for over 100 years However, until recently, there were few consensus periodontal pathogens. Some of the reasons for the uncertainly in defining periodontal pathogens have been reviewed In brief; investigators have been faced with the task of discriminating pathogens from host-compatible species in the following special circumstances:

■ Over 300 species may be cultured from the periodontal pockets of different individuals, and 30 to 100 species (any of which may be the pathogen) may be recovered from a single site

• Many of the species in pockets are difficult or impossible to culture and difficult to identify.

• The physical constraints of a pocket make it difficult to obtain a representative sample from the pocket: that is, a sample that contains the pathogen and low numbers of contaminating specie;

• Sites within subjects do not appear to be actively progressing at all times, so that the time of sampling may play as critical a role as the site of sampling in understanding the pathogenesis of disease.

• There appear to be multiple destructive periodontal diseases that, for the most part, cannot be differentiated on a clinical basis. Thus, disease types may be misclassified

and inappropriately pooled.

• Different sites, in the mouth may break down as a result of different pathogens and sites may show activity due to one pathogen at one time and second pathogen at a later time.

• Opportunistic species may grow as a result of disease rather than being the cause. Their levels may increase concomitant with or after those of the true pathogens, and they may thus be difficult to distinguish experimentally

• It seems likely that some of the infections in periodontal pockets are mixed infections. If it is difficult to evaluate the role of single species in disease etiology, it becomes even more difficult to evaluate all possible pairs or larger mixtures of species.

•Pathogens may be carried in low numbers mouths free of destructive periodontal disease (the so-called carrier state), making their role in disease more difficult to evaluate. Strains of putative pathogens may differ in virulence. Avirulent clonal types might be detected in periodontally healthy subjects, whereas virulent clonal types might be present in subjects with periodontal disease. An inability to distinguish virulent from avirulent clonal types would; impede understanding.

Suggestions have been made that more virulent strains of a species may harbor bacteriophages or plasmids that might confer virulence properties. Failure to recognize such genetic elements would limit the understanding of the relationship of either the genetic element, or the species, to periodontal disease progression.

THE ECOLOGY OF THE MOUTH

The mouth harbours many microorganisms in an ecosystem" of considerable complexity that has not been fully 'investigated yet and is far from completely understood. Until quite recently the mouth was regarded as a single habitat for microorganisms but is now realized that the teeth, gingival crevice, tongue, other mucosal surfaces and saliva all form different habitats or sites where microorganisms multiply. Each habitat contains its own characterstic population often with many microbial species. These species may complement or compete with others in same population and thus the oral flora is a dynamic entity affected by many changes throughout the life of the host.

DEVELOPMENT OF THE ORAL FLORA

BIRTH

The mouth of the full-term foetus is usually sterile, although organisms which are only transient may be acquired from the vagina at birth. The mouth of the newborn baby rapidly acquires organisms from the mother and also from the environment. Several streptococcal and staphylococcal species may be isolated, together with coli forms, lactobacilli permanent *Bacillus* spp, *Neisseria* spp and yeasts. *Streptococcus salivarius* is the most common isolate from the mouths of young, babies and together with *Staphylococcus albits*, *Neisseria* spp and *Veillonella* spp they form the pioneer community. Occasionally *Candida albicans* multiplies rapidly in the mouth and the low pH it prevents the normal growth of other commensals.

INFANCY AND EARLY CHILDHOOD

The infant comes into contact with an ever-increasing range of microorganisms and some of these will become established as part of the commensal flora of the individual. Commensal organisms in other sites of the body and organisms from the environment will be presented to the oral cavity and some will become established. The eruption of deciduous teeth provides a different surface for microbial attachment and this is characterised by the appearance of *Streptococcus sanguis* and *mutans* as regular inhabitants of the oral cavity. With increasing numbers of teeth and changes in diet the overall proportions of organisms in the mouth will change. A few anaerobes become established but as there is no deep gingival crevice the numbers remain small. Actinomycetes, lactobacilli and *Rothia* are found regularly

ADOLESCENCE

Perhaps the greatest increase in numbers of organisms in the mouth occurs when the permanent teeth erupt. These teeth have deep fissures in their surfaces that do not readily wear down. The interproximal spaces are much larger than in the deciduous dentition as the teeth have a more pronounced 'neck' at the amelo-cemental junction. The gingival crevice is deeper than in the deciduous dentition and allows for a great increase in anaerobic organisms. *Bacteroides* spp become established in large numbers as well as *Leptotrichia* spp, *Fusobacterium* spp and spirochetes. The lesions of 'dental

caries will create a new environment in which some organisms especially streptococci will flourish. In ecological terms the oral flora of late adolescence and early adulthood, before the loss of teeth, is the climax community. ADULTHOOD

The complexity of the oral flora of the adult is perhaps its chief characteristic. Varying amounts of dental plaque may be present and the degree of chronic periodontal disease will also govern the numbers and types of microorganisms found. Carious lesions and unsatisfactory restorations will provide environments for local accumulations of bacteria. Most studies of the adult oral flora show that considerable variation occurs among individuals in total numbers and proportions of many species of bacteria; indeed there may be variation within one individual when sampled on several occasions.

Consistent with the trends seen in adolescence there is an increase in the. *Bacteroides* spp and spirochetes with advancing periodontal disease and maturity of dental plaque. Superficial plaque contains many streptococci, mostly *Streptococcus mutans, minor (mitis)* and *sanguis.* Actinomycetes and other Gram-positive and Gram-negative filaments *of* uncertain taxonomic position are also regularly isolated.

As teeth are lost so the numbers of available sites for microbial colonisation decrease; the numbers of bacteria 'decrease and several species diminish disproportionately in numbers. Edentulous patients harbour few spirochetes or bacteroides but their carriage of yeasts increases. Yeasts are normally found on the dorsum of the tongue and in the upper buccal sulcus. Dentures provide a protected environment in which yeasts can multiply, covering the hard palate and the acrylic denture surface.

PERIODONTAL PATHOGENS

The world workshop in periodontology(consensus report 1996) designated A.actinomycetemcomitans, P.gingivalis and B.forsythus as periodontal pathogens.

A. actinomycetemcomitans:

The morphological and cultural characteristics: of A.actinomycetemcomitans were first described by Klinger . The first isolates of this gram-negative, facultatively

anaerobic rod. recovered from cervicofacial actinomycosis, were given the name Bacterium actinomycetemcomitans. This name was changed twice, first by Lieske in 1921 to Bacterium comitans and later by Topley & Wilson in 1929 to Actinobaciilus actinomycetemcomitans. The genus name refers to the star shaped inner structure sometimes seen in colonies on selective medium and to the short rod or bacillary-shaped form of the cell; The specific epithet means "with actinomyces", referring to its close association with Actinomyces israelii in actinomycotic lesions. A. actinomycetemcomitans had been detected in lesions caused by A. israelii as densely packed gram- negative cocco-bacilli

Biochemical properties of A. actinomycetemcomitans

Slots studied 135 biochemical characters in 6 reference strains and 130 strains of A. actinomycetemcomitans freshly isolated from the oral cavity. Upto then biochemical studies had been few and somewhat conflicting. All the isolates were small, nonmotile capnophilic gram-negative rods that did not require X or V factor for growth. They all decomposed hydrogen peroxide, were oxidase negative and benzidine positive, reduced nitrate, produced strong alkaline and acid phosphatases and fermented fructose, glucose and mannose. Variable fermentation results were obtained with dextrin, maltose, mannitol and xylose. Some isolates produced small amounts of gas. Hydrogen sulfide was not generated.

Characters useful for distinguishing A.a from the closely related H. aphrophilus included the catalase reaction, fermentation of lactose, starch, sucrose and trehalose, and sodium fluoride resistance, as well as ability of H. aphrophilus to produce p-glucosidase and p-galctosidase. A. actinomycetemcomitans was distinguished from Haemophilus species requiring X and V factors by its ability to grow in absence of these factors.

NOT ALL ACTINOBACILLUS ACTINOMYCETEMCOMITANS ARE CREATED EQUAL: THE SPECIFIC PLAQUE HYPOTHESIS REVISITED

The gram-negative facultative anaerobe *Actinobacillus actinotnycetemcmiitans* is implicated as a pathogen in several fonns of aggressive and chronic periodontitis, most

notably localized aggressive periodontitis. It is one of a few periodontal microorganisms that are strongly implicated in the etiology of periodontitis, providing support for the Specific Plaque Hypothesis. *A. actinomycetemcomitans* possesses the ability to kill human leukocytes through the production of a 116-kDa protein toxin termed a *leukotoxin.* Because leukocytes are critical to an effective host response against periodontal pathogens, the ability of this raicrooiganism to kill leukocytes is an impoitant mechanisni in evading host defenses. Early studies indicated that not all strains of *A. actinomycetemcomitans* demonstrated the leukotoxic properties.[2] Recent investigations have revealed a molecular basis for this variability in leukotoxin expression and led to new insights on the role of specific strains of *A, actinomycetemcomitans* in periodontitis.

The Leukotoxin of A. actinomycetemcomitans

The *A. actinomycetemcomitans* leukotoxin (LtxA) is a member of a family of pore-forming toxins, characterized by a series of glycine-rich repeats in the C-terminal portion of the protein that are involved in cation binding and appear to be essential to toxin activity. This family of toxins has been designated as RTX (repeat in toxin) toxins, and they are produced by several pathogenic, gram-negative species. Other RTX toxins include the leukotoxin of *Pasturella naemoiytica,* a respiratory pathogen of cattle, as well as hemolysins of pathogenic *Escherichia coli* and *Bordetella pertussis,* the causative agent of whooping cough. A high degree of specificity in the cells affected by the *A. actinomycetemcomitans* LtxA was evident in early studies. The affected target cells include human polymorphonuclear leukocytes (neutro-phils or PMNs), monocytes and lymphocytes. Human platelets, fibroblasts, and endothelial and epithelial cells are resistant to the effects of the LtxA.[71 "B] The target cell susceptibility is a result of cell surface expression of the #2-integnn molecule, lymphocyte function-associated antigen 1 (LFA-1). The identification of LFA-1 as the receptor for LtxA suggests that killing is a receptor-mediated process.

Mechanism of Leukotoxin Action

Evidence exists for two LtxA-mediated mechanisms of cell death; necrosis and apoptosis. Exposure of neu-trophils and monocytes/macrophages to strains that pro-duce large amount of LtxA results in killing within a relatively short period of time

(Fig. 9-1, panels A-C).[2] This cell death is thought to result from the ability of LtxA to form pores in the membrane of target cells, leading to osmotic lysis caused by water influx into the cell.[45] In contrast, prolonged exposure of lymphocytes and NK tells to LtxA results in the induction of apoptosis, a programmed sequence of cellular alteration progressing lo cell death.[41]"""" There is evidence that Sower concentrations of LtxA result in apopiosis whereas higher concentrations result in necrosis.[1]" It has been hypothesized that the LtxA molecules in high concentration fuse to form large pores in the target cell membrane, resulting in more rapid necrotic destruction.

Molecular Characterization of the Leukotoxin Genes

The gene encoding the *A. actinomycetmecomitans* leukotoxin, *ItxA,* is part of an operon of four genes in the sequence *itxC, itxA, itxB,* and taD . This operon structure of C, A, B, and D genes in sequence is characteristic of the RTX family of toxins. Considerable sequence homology exists between the individual genes from these different organisms, suggesting a common evolutionary origin and function.[41]"' As is characteristic of genes clustered in an operon, these genes are all related to leukotoxin function. The A gene encodes the leukotoxin itself and is produced in an inactive "pro-toxin" state. The C gene product, *ItxC,* is required to activate the pro-toxin. In the case of the *E. coli* hemolysin, this process involves a chemical modification in which fatty acids are linked to two sites on the leukotoxin protein.[69] Based on the similarity between the C genes and gene products, the *ItxC* gene product of *A. actinomycetemcomitans* also is thought to function in leukotoxin activation. The B and D genes are typically involved in secretion of the leukotoxin from the bacterial cell, and studies of the *ttxB* and taD genes of *A. actinomycetem-comitans* suggest similar functions.[2243] For many years, it was thought that the ieukotoxin of *A, actinomycetemcomitans* remained associated with the bacterial cell and was not secreted into the external environment. Recent studies have revealed that certain strains in their early growth phases secrete an abundant amount of leukotoxin into the environment.[43] FuTther studies of the factors governing the cellular retention or release of the leukotoxin may shed more light on the role of these properties in palhogenesis.

Molecular Basis of Variability in Leukotoxin Production

It has been long recognized that some strains of *A, actinomycetemcomitans* are highly toxic and produce high levels of leukotoxin, whereas other strains are weak or minimally toxic and produce low levels of leukotoxin. A key discovery in the study of the leukotoxin production in *A. actinomycetemcomitans* was the variation in the DNA sequence of the leukotoxin promoter region.[92"] Bacterial promoters consist of specific segments of DNA that provide a recognition and binding site for the enzyme RNA polymerase, which is responsible for RNA (ribonudeic acid) synthesis. Different promoters vary in their level of transcription, leading to differences in the level of mRNA and thus differences in the amount of protein product generated. The regulation of gene expression based on the amount of mRNA produced is referred to as *transcrip-tional control.* Investigation of the DNA sequence upstream of the *ItxC* gene revealed that the highly toxic strains have a deletion of 530 base pairs (bp) of DNA as compared with the minimally toxic strains . Analysis of mRNA revealed the presence of two promoters functioning in the highly toxic strains (see Fig. 9-2, *A,* PI and *V2),* But in the minimally toxic strains a single promoter is responsible for initiating transcription . This latter promoter (P3) resides within the 530 base pair region that is missing in the highly toxic strains. Studies of the regulation of transcription further differentiate the different promoters. The PS promoter is regulated by the level of oxygen present during bacterial growth, with increased mRNA, increased leukotoxin expression, and a 3- to 4-fold increase in to.xicity when the bacterium is grown under anaerobic conditions. In contrast, oxygen concentrations have no effect on the PI or P2 promoters. The amount of mRNA and leukotoxin produced and the resulting toxicity are substantially greater in the highly toxic strains using the P1 and P2 promoters, regardless of the environmental conditions, as compared with the minimally toxic strains using the P3 promoter. It is hypothesized that the highly toxic strains may have evolved from a minimafly toxic strain by the deletion of this DNA in the promoter region.

The leukotoxin is considered *an* important virulence determinant of *A. actiuornycelemamiitans* because it allows the microorganism to neutralize host defense mechanisms. The interaction of highly versus minimally toxic strains with neutrophils is demonstrated. The highly toxic strain rapiidly induces degenerative changes and then lysis of the neutrophils . In contrast, the minimally toxic strain does not induce lysis

of the neutrophil, but rather the bacterial cells are phagocytosed and phagolysosomes containing the bacterial ceils are evident microscopically . The presence of the 530-bp deletion in the DNA sequence has provided a marker foi distinguishing the highly versus minimally leukotoxic strains, which can be easily detected using PCR techniques. The occurance of the highly leukotoxic strain varies widely in different geographic regions as well as in different racial groups.[2i] In addition, in a population of children at risk for the development of localized aggressive periodontitis, those who harbored the highly leukotoxic strain were found to be more likely to develop disease.

Summary

The significance of the findings related to leukotoxin expression ranges from theoretic to practical.[64] The theoretic implications relate to the clear demonstration that not all *A, actinomycttemcanritans* arc created equal, but rather that some strains are more virulent than others The specific plaque hypothesis states that disease is associated with specific bacterial species. The findings described here suggest that the specific plaque hypothesis should be revised to state that disease is associated with specific strains of a given species. It is likely that most, if not all, recognized periodontal pathogens demonstrate differences in phenotypic properties related to their ability to cause disease. Further investigation at a molecular Jevel will undoubtedly continue to reveal the genetic basis for variability in virulence properties among pathogenic strains. Practical implications relate lo microbial monitoring of pertodontitis patients as well as assessments of risk in susceptible individuals. Current practices of microbial testing do not differentiate the highly toxic versus minimally toxic *A. actinomycetein-comihms* strains. This can lead to a situation where an individual with a minimally toxic strain might be inappropriately considered at high risk for disease development or progression. Furthermore, in clinical trials in which the presence of *A. actinomycetemcomitans* is evaluated as a risk factor for development or progression of disease, the inclusion of minimally toxic strains may dilute and essentially mask a stronger association of the highly leukotoxic strain with disease. Clarification of specific bacterial virulence factors that contribute to pathogene-sis and development of the tools or piobes needed to identify strains possessing virulence factors will enhance our ability to both prevent and manage periodontal diseases.

Family Bacteroidaceae:

The family bacteroidaceae contain many important human and animal pathogens and currently comprise a large collection of genera of obligatory anaerobic, gram negative, non spore forming rods the family was described in the first edition of Bergey's manual in 1923 with bacteroides as type genus. Over the years, major taxonomic revisions have taken place within the Bacteroides which eventually led to the proposal of these genera. Bacteroides, Fusobacterium and Leptotrichia.

Classification was based largely on the end-product profile. Hence, two of these taxa Fusobacterium and Leptotrichia, characterized by their low mol % guanine+cytosine content, were differentiated by the production of mainly lactic acid by the latter, while Fusobacterium produce acetic and butyric acids as major end-products of metabolism.

Recent phylogenetic analyses confirmed the close affinity between these two genera and surprisingly showed that they were more closely related to gram-positive bacteria than Bacteroides The description of the genus Bacteroides was based on the production of mixed acid end products and thus, over many years became a repository for anaerobic species that could not be accommodated elsewhere.

In 1989 the genus was redefined and two new genera, Porphyromonas and Prevotella, were proposed. At present more than 15 genera have been described for taxa that were formerly classified as Bacteroides. However, it should be noted that the taxonomic restructuring of this group continues based on phylogenetic relationships.

Emergence of the genus Porphyromonas, emendation taxonomic position of P. gingivalis

The genus Porphyromonas and, in particular the species P. gingivalis is relatively new delineated from Bacteroides melaninogenicus. Castellani & Chalmers suggested the placement of gram-negative, nonsporeforming, non-motile anaerobic rods in the genus Bacteroides. Two years later, Oliver & Wherry reported morphologically similar bacteria from a variety of infections that produced black-pigmented colonies on blood agar and considered them members of this genera. The black pigment was thought to be melanin and extracts of P. gingivalis resulted in the recognition of different serogroups among human isolates, two of which correlated with a possible increase in pathogenic potential. P gingivalis isolates delineated in group A were mainly

from healthy patients and included the low-virulence reference strain ATCC 33277, whereas those from patients with severe periodontitis belonged to group B, which included the more virulent strain W50

BIOCHEMICAL PROPERTIES

P. gingivalis is an asaccharolytic, anaerobic species and consequently colonizes sites where the oxygen tension is low but nitrogenous substrates are present in abundance. Thus, the isolation of strains from saliva or the mucous membranes of the tongue and tonsils may represent a temporary transition phase for this species. The subgingival ecosystem provides an ideal environment for this species, as the redox potential is low and is lowered still further in disease while endogenous nutrients are both rich in peptides and amino acids; The bacterium possesses an electron transport system in which protoheme (the prosthetic group of cytochromes) and menaquinones (containing nine isoprenc units) are the major electron carriers. Both these compounds are likely to be present within the periodontal pocket. It has been demonstrated that aspartate and its corresponding amide stimulate growth and are catabolized via oxaloacetate, malate and fumarate to yield succinate (the succinate pathway). Fumarate acts as electron sink in accepting reducing equivalents from various electron donors such as NADH. It is possible to culture *P. gingivalis* in the absence of exogenous menadione; hence it has been assumed that this species can biosynthesize menaquinones from simple precursors in the medium. The same is not true for heme and, in general, cultures will only survive for a limited number of generations in the absence of a source of heme.

Bacteroides forsythus

The third consensus periodontal pathogen, B. *forsythus,* was first described in 1979 (Tanner et al) as a "fusiform" *Bacteroides* This species was difficult to grow, often requiring 7-14 days for minute colonies to develop. The organism is a Gram-negative anaerobic, spindle-shaped, highly pleomorphic rod. The growth of the organism was shown to be enhanced by co-cultivation with F. *nucleatum* and indeed commonly occurs with this species in subgingival sites (Socransky et al. 1988). The species was shown to have an unusual requirement for N-acetylmuramic acid. Inclusion of this factor in culture media markedly enhanced growth. The organism was found in higher numbers in sites of destructive periodontal disease or periodontal abscesses than in gingivitis or healthy sites. In addition, *B.forsythus* was detected more frequently and in

higher numbers in active periodontal lesions than inactive lesions. Further, subjects who harbored *B.forsythus* were at greater risk for alveolar bone loss, attachment loss and tooth loss compared with subjects in whom this species was not detected. This species has been shown to produce trypsin like proteolytic activity (BANA test positive, Loesche et al. 1992), methylglyoxal and induce apoptotic cell death

Initially. *B.forsythus* was thought to be a relatively uncommon subgingival species. However, the studies of Gmur et al (1989) using monoclonal antibodies to enumerate the species directly in plaque samples, suggested the species was more common than previously found in cultural studies and its levels were strongly related to increasing pocket depth. Lai et al. (1987) corroborated these findings using fluorescent-labeled polyclonal antisera and demonstrated that B. *forsythus* was much higher in subgingival than supragingival plaque samples. Data of Tanner et al. (1998) suggested diet *B.forsythus* was a major species found at sites that converted from periodontal health to disease. B. *forsythus* was found at higher levels at sites which showed breakdown after periodontal therapy than sites which remained stable or gained attachment. B. *forsythus* has also been shown to be decreased in frequency of detection and counts after successful periodontal therapy including SRP, periodontal surgery or systemically administered antibiotics. Successful treatment of peri-implantitis with local delivery of tetracycline was accompanied by a significant decrease in the frequency of detection of *B.forsythus.*

Studies using checkerboard DNA-DNA hybridization techniques to examine subgingival plaque samples confirmed the high levels *of B.forsythus* detected using fluorescent-labelled antisera and demonstrated that B. *forsythus* was the most common species detected on or in epithelial cells recovered from periodontal pocket: It was infrequently detected in epithelial cell samples from healthy subjects. Double-labelling experiments demonstrated that B. *forsythus* was both on and in periodontal pocket epithelial cells indicating the species ability to invade. Listgarten et al. (1993) found that the species most frequently detected in "refractory" subjects was *B.forsythus.* Serum antibody to *B.forsythus* has been found to be elevated in a number of periodontitis patients and was often extremely elevated in a subset of refractory periodontal disease subjects.

The role of this species in periodontal diseases has been clarified by studies in numerous laboratories involving non-cultural methods of enumeration such as DNA

probes, PCR or immunologic methods. For example, Grossi et al. (1994, 1995) considered B. *forsythus* to be the most significant microbial risk factor that distinguished subjects with periodontitis from those who were periodontally healthy

DESTRUCTION AT THE HOST TISSUE INTERFACE: THE PROTEASES OF PORPHYR0M0NAS GINGIVALIS

P gingivalis is a periodontal microorganism that is strongly associated with chronic and aggressive forms of periodon-tai disease in humans. In addition, this gram-negative anaerobe has been shown to be pathogenic in nonhuman primate and rodent models of periodontitis. Investigations in the laboratory have identified numerous properties of *P. gingivalis* that may account for its ability to cause disease, and preeminent among these is the ability of *P. gingivalis* to elaborate a variety of

proteolytic enzymes. Investigations during the last 2 decades demonstrated that a wide variety of protein substrates are degraded toy *P. gingivalis* proteases. This led to a period of considerable confusion regarding the number and activity of different proteases. Molecular analyses have now confirmed the presence of a relatively limited number of protease genes and clarified the relationships among genes characterized from different strains.

It is noteworthy that *P. gingivalis* proteases are not inactivated by host proteinase inhibitors. Rather, evidence exists that the *P. gingivalis* proteases inactivate or degrade the host proteinase inhibitors.[57] This may lead to an imbalance in the normal host mechanisms of tissue turnover, contributing further to host tissue destruction. The proteases of *P. gingivalis* are important in the metabolism and ecology of the bacterium itself, but they additionally act at the host-parasite interface to contribute to patho-genesis through host tissue degradation and modulation of host defense mechanisms. Molecular analyses have been used to define the genetic basis of protease production and delineate the mechanisms by which the proteases may contribute to host tissue destruction. In addition, genetic approaches to specifically inactivate protease genes have been used to demonstrate that proteases play an important role in the virulence of *P. gingivalis,*

P. gingivalis Proteolytic Activity

P. gingivalis is unable to break down or degrade sugar substrates as a source of energy or to take up and use free amino acids as metabolic building blocks. Rather, P. *gingivalis* relies on its ability to degrade proteins into short peptides that are taken in and used metabolically in the generation of energy and as sources of carbon and nitrogen. In the periodontal environment, the host tissues provide an abundant source of proteins. For example, a major protein constituent of the periodontal tissues is collagen, and *P. gingivalis* possesses the proteolytic activity to degrade collagen into peptide components that it can use. The bacterial collagenase produced by *P. gingivalis* (PrtC), host cell collagenases, or probably both contribute to collagen degradation. Several pepti-dases found on the cell surface of *P. gingivalis* are suffi-cient to completely degrade the collagen fragments. These includ^ipeptidyl peplidase IV (DPPIV) and prolyl trlpeptidyl peptidases (PtpA), proteases that generate di-and tri-peptide fragments, which may be transported into the bacterial cell. Another group of *P. gingivalis* proteases, the gingipains (discussed below), also are capable of degrading collagen fragments. The degradation of collagen and other host tissue molecules supports *P. gingi-valis* metabolically but also contributes to pathogenesis because of the resulting host tissue damage.

Molecular Characterization of the Gingipain Protease Genes

The most intensely studied enzymes of *P. gingivaiis* are a group of related proteases known as *gingipains.* These proteases occur in rnultiple forms that are found extracellu-lariy or on the bacterial cell surface and in some cases are associated with protein regions or "domains" involved in adherence properties . The gingipains specifically cleave proteins at the peptide bond following argi-nine residues {Arg-gingjpafns or Rgps) 01 lysine residues (Lys-gingipain or Kgp). Molecular studies revealed that the Arg-gingipains are encoded by two genes, *rgpA* and *rgpB,* whereas the Lys-gingipain is encoded by a single gene, *kgpA.* Analysis of the *rgpA* DNA sequence indicates that the piotein product initially consists of three distinct domains: a propeptide domain at the ammo-terminal end of the protein that is cleaved off when the protease is activated, a protease domain, and a car boxy-terminal adhesin domain . The propeptide and protease domains are very closely related to those of the second Arg-gingipain gene, *TgpB.* In contrast, *rgpB* lacks the ad-hesin domain. The Lys-gingjpain gene *kgp* is similar in organization to *rgpA,* with propeptide, protease, and

ad-hesin domains. The ammo acid sequence of the Kgp protease domain demonstrates only 22% identity to that of RgpA, with key catalytic regions that are identical and substantial regions of unrelated sequence that may account for the differences in substrate specificity between the two enzymes. The transiational protein products of the *rgp* genes undergo posttranslational modifications, including cleavage and addition of carbohydrate groups, to yield multiple forms (termed *isoforms)* of the protease . Interestingly, the adhesin domains found in RgpA and Kgp share stretches of sequence that are closely related to adhesin domain sequences found in a hemag-glutinin (HagA) and an outer membrane receptor protein (Tla) of *P. gingivalis.* It has been demonstrated that the adhesin domain mediates adherence of the protease-ad-hesin complex to connective tissue molecules, including fibrinogen, fibronectin, and laminin. Furthermore, the adhesin-protease complex is about twice as effective in degrading nbrinogen and fibrin as compared with the protease lacking the adhesin.[31] Adherence is an important component of pathogenesis, and the combination of adherence and protease activity is likely to facilitate the process of tissue degradation.

Functional Studies of the Gingipain Proteases

The gingipains of *P. girtgivalis* are capable of disrupting normal host systems in a way that contributes to the growth and virulence of the bacterium and destruction of the host tissues .The ability of gingipains to stimulate the release of bradykinin, resulting in increased vascular permeability, provides a mechanism to explain the increased crevicular fluid flow evident at sites of inflammation, a condition that improves the nutrient supply for resident microorganisms. The host immune response may be neutralized by the gingipains because they degrade the host cell receptor for LPS (CD14), the proinflammatory cytokines such as 1L-10, 11,-6, and the chemokine IL-8. In addition, the effects on neu: trophil function and degradation of host inflammatory mediators alter the normal host inflammatory responses to the bacterial assault. The gingipains appear to both stimulate and inhibit the host immune response wiih regard to neutrophil function. For example, the soluble Rgps and Kgp cleave 1L-8 in a manner that makes this proinflammatory cytokine more active in the recruitment of neutrophils. However, the membrane-associated Rgps are able to abolish IL-8 activity by completely degrading the molecule. This has led to the hypothesis that *P. gingivalis* has proinflammatory effects at long distances mediated by soluble proteases but antiinflammatory effects at close range mediated by membrane-

associated proteases. Inherent in this hypothesis is the premise that *R gingivalis* benefits from the presence of neutrophils in the area because the neutrophils release a wealth of proteolytic enzymes that undoubtedly contribute to tissue protein degradation and thus assist in the acquisition of nutrients for the bacterium. However, neutrophil function is thought to be inhibited in close proximity to *P. gingivalis* cells, by virtue of the membrane-associated proteases on the bacterial cells or vesicles released into the bacterial cell's immediate surroundings, to protect the bacterium from phagocytosis. Mechanisms for the inhibition of neutrophil function include the degradation of IL-8 by membrane-associated Rgps as well as molecules (e.g., complement component C3) and receptors (neutrophil C5a receptors) involved in opsonization and phagocytosis.

Inactivation of the Gingipain Genes

Molecular studies often focus initially on isolating a particular gene of interest by cloning and expressing that gene in a foreign bacterial host system such as *E. coli*. This approach has been invaluable in isolating and characterizing genes and their products. Another powerful tool in molecular studies is to inactivate, or "knock out," specific target genes at the molecular level. This is typically achieved by inserting an unrelated "marker" gene, such as an antibiotic resistance gene, into the coding sequence of the target gene on the chromosome of the native microorganism. Transcription of the target gene is disrupted, and the presence of the "marker" antibiotic resistance gene is easily assessed by growth of the bacterium on media containing the relevant antibiotic. The resulting bacterial strain is termed an *isagenk mutant.* These mutants differ from the parental strain by the disruption of one, or in some cases several, defined gene(s) and provide an important technique for assessing the role of the gene product in the native host cell. *P. gingi-valis* mutants in the *rgpA* and *rgpR* genes confirmed that both genes encode Arg-gingipains . A mutant strain in which both the genes have been inactivated indicates that these two genes account for all of the Arg-gingipain activity found in *P. gingivalis.* Similarly, the properties of an isogenic mutant in *kgp* indicates that this gene accounts for the Lys-gingipain activity.[59] The loss of hemagglutination in mutants with the *rgpA* or *kgp* genes inactivated supports a role for the protease-associated adhesin domains in hemagglutination.

Analysis of the gingipain isogenic mutants further confirms studies on the role of these proteases in bacterial nutrition, tissue destruction, and modulation of the host immune response. The mutant with all three gingipain genes *{rgpA, rgp$,* and *kgp)* inactivated demonstrated a complete loss of extracellular proteolytic activity and diminished growth on complex media, presumably because of an inability to degrade proteins for use in bacterial metabolism.[67] Inactivation of the *rgpA.* gene specifically resulted in loss of the ability of the bacterium to degrade type 1 collagen, a major component of the periodontal tissues.[71] Mutations in both *rgpk* and rgpB were associated with alterations in the bacterial-neutrophil interaction. The supernatants taken from cultures of *P. gingivalis* normally inhibit neutrophil function. Strains with either *rgpA* or *rgpQ* inactivated partially lose the ability to Inhibit neutrophil function, whereas inactivation of both *rgpA* and *rgpB* results in almost complete loss of the ability to inhibit neutrophil function. In addition, the *rgpA* mutant is less resistant to neutrophil phagocytosis.[65] This mutant demonstrated a loss in the ability to degrade the complement factor C3 and an increase in the accumulation of iC3b on the bacterial cell surface. Thus the loss of Arg-gingipain activity is associated with a loss in the ability of the bacterium to disrupt the processes of opsonization and phagocytosis.

Several unexpected findings also have emerged from the studies of the gingipain isogenic mutant strains. Inactivation of the *rgp* genes is associated with altered expression or loss of the bacterial nmbriae normally found on the *P. gingivaiis* cell surface. The iim-briae are important in adherence of the bacterial cells, as reflected in the alterations in adherence properties observed in the *rgp* mutant strains. Subsequent investigation revealed that Arg-gingipain plays an important role in the processing of prefimbrilin to form nmbrilin, a major component of the bacterial nmbriae. Fimbrial expression is important in the virulence of *P. gingivalis,* underscoring again the role of Arg-gingipain activity in pathogenesis. A second unexpected finding was that in-activation of *kgp* results in a loss of the black pigmentation of the *P. gingivalis* colonies as well as altered adsorption of hemoglobin and heme accumulation. *P. gingivalh* colonies normally turn black when grown on blood agar as the cells accumulate an oxidized form of heme, hemin. The hemin appears to be derived from hemoglobin and provides an important nutritional source of iron for the bacterium. Subsequent studies revealed that Lys-gingipain is able to cleave human hemoglobin and thus appears to function as a hemoglobinase in the acquisition of heme and iron. Interestingly, the hemoglobin receptor protein of P. *gingivalis* is

encoded as part of the adhesin domain associated with the *kgp, rgpA,* and *hagA* genes. The physical proximity of the hemoglobin receptor and the hemoglobinase in the protein product presumably facilitates this aspect of nutrient aquisition. Hemin levels are known to be important in the virulence properties of P. *gingivalis.*[sl] The unexpected findings described here illustrate the importance of investigating gene function, not just in a foreign host System such as *E. coli* but in the native host cell (in this case, *P. gingivalis),* to fully understand the role of any particular gene or gene product in pathogenesis.

SPIROCHETES

Spirochetes are Gram-negative, anaerobic, helical-shaped, highly motile microorganisms that are common in many periodontal pockets. Clearly, a spirochete has been implicated as the likely etiologic agent of acute necrotizing ulcerative gingivitis by its presence in large numbers in tissue biopsies from affected sites (Listgarten & Socransky 1964, Listgarten 1965).

The role of spirochetes in other forms of periodontal disease is less clear. The organisms have been considered as possible periodontal pathogens since the late 1800s and in the 1980s enjoyed a resurgence of interest for use as possible diagnostic indicators of disease activity and/or therapeutic efficacy. The major reason for the interest in this group of organisms has been their increased numbers in sites with increased pocket depth. Healthy sites exhibit few, if any, spirochetes, sites of gingivitis but no attachment loss exhibit low to moderate levels, while many deep pockets harbor large numbers of these organisms.

The major difficulty encountered in defining the role of spirochetes has been the difficulty in distinguishing individual species. At least 15 species of subgingival spirochetes have been described, but in most studies of plaque samples, spirochetes are combined in a single group or groups based on cell size; i.e. small, medium or large- Thus, while there may be pathogens among the spirochetes, their role may have been obscured by unintentionally pooling their numbers with non-pathogenic spirochetes.

This would be similar to combining in a single count, organisms with coccal morphologies, such as P. gingivalis, Veillonella parvula and Streptococcus sanguis. In spite of the limitations of combining spirochetes into a single morphogroup, spirochetes have been related with increased risk at a site for the development of gingivitis and periodontitis.

The need to evaluate the role of individual species of spirochetes in periodontal diseases is reinforced by studies of serum antibody responses to different species. When be realistic based on their detection in plaque samples by immunologic, PCR or DNA probe techniques. Indeed, enumeration of even uncultivable spirochete taxa is possible using oligonucleotide probes (Tanner et al. 1994) or specific antibody as described above.

PREVOTELLA INTERMEDIA /PREVOTELLA NIGRESCENS

P. intermedia is the second black-pigmented Bacteroides to receive considerable interest. The levels of this Gram-negative, short, round-ended anaerobic rod have been shown, to be particularly elevated in acute necrotizing ulcerative gingivitis, in certain forms of periodontitis and in progressing sites in chronic periodontitis and has been detected by immunohistological methods in the intercellular spaces of periodontal pocket biopsies from rapidly progressive periodontitis subjects.

Isolates of this species can induce alveolar bone loss in rats. Persistence of P. intermedia /nigrescens after standard mechanical therapy has been shown to be associated with a large proportion of sites exhibiting bleeding on probing (Mombelli et al 2000). Berglundh et al. (1998) demonstrated that improved clinical parameters after the use of mechanics therapy and systemically administered amoxicillin and metronidazole were associated with decrease periodontal pathogens including P. intermedia. Successful treatment of peri-implantitis with local delivery of tetracycline also significantly decreased the frequency of detection of P. inter-media/nigrescens.

This species appears to have a number of the virulence properties exhibited by P. gingivalis and was shown to induce mixed infections on injection in laboratory animals. It has also been shown to invade oral epithelial cells in vitro. Elevated serum antibodies to this species have been observed in some but not all subjects with refractory periodontitis. Strains of P. intermedia" that show identical phenotypic traits have been separated into two species, P. intermedia and P nigrescens (Shah & Gharbia 1992). This distinction makes earlier studies of this "species" difficult to interpret since data from two different species may have been inadvertently pooled. However, new studies which discriminate the species in subgingival plaque samples might strengthen the relationship of one or both species to periodontal disease pathogenesis.

FUSOBACTERIUM NUCLEATUM

Gram-negative, anaerobic, spindle-shaped rod that has been recognized as part of the subgingival microbiota for over 100 years (Plaut 1894, Vincent 1899). This species is the most Common isolate found in cultural studies of subgingival plaque samples comprising approximately 7-10% of total isolates from different clinical conditions F.nucleatum is prevalent in subjects with periodontitis and periodontal abscesses.

Successful treatment of peri-implantitis with local delivery of tetracycline was associated with a significant reduction in frequency of detection in several species including F. nucleatum. Invasion of this species into human gingival epithelial cells in vitro was accompanied by an increased secretion of IL-8 from the epithelial cells. The species can induce apoptotic cell death in mononuclear and polymorphonuclear cells and cytokine, lactase and oxygen radical release from leukocytes

Although there were differences detected in levels of this spedes between active and inactive periodontal lesions, the differences may have been minimized by the inadvertent pooling of subspecies of F. nucleatum. Support for this contention may be derived from the antibody responses in subjects with different forms of periodontal disease to different homology groups of F. nucleatum (Tew et al, 1985b). It is anticipated that a clearer understanding of the role of F. nucleatum will be achieved when subspecies such as F. nucleatum ss nucleatum, F. nucleatum ss polymorphum, F. nucleatum ss vincentii and F. periodonticum are individually evaluated for their association with disease status and progression.

CAMPYLOBACTER RECTUS

C. rectus is a Gram-negative, anaerobic, short, motile vibrio. The organism is unusual in that it utilizes H_2 or formate as its energy source. It was first described as a member of the "vibrio corroders", a group of short nondescript rods that formed small convex, spreading" or "corroding" (pitting) colonies on blood' agar plates. These organisms were eventually shown to include members of a new genus Wolineila (most species have been redefined as Campylobacter), and Eikenella corrodens. C. rectus has been shown to be present in higher numbers in disease sites as compared with healthy sites and it was found in higher numbers and more frequently in sites exhibiting active periodontal destruction-or converting from periodontal health to disease. In addition, C. rectus was found less frequently and in lower numbers after successful periodontal therapy or

treatment of peri-implantitis with local delivery of tetracycline Mombelli et al. C rectus was also found in combination with other suspected pathogens in sites of subjects with refractory periodontal disease like Aa, C.rectus has been shown to produce a leukotoxin. These are the only two oral species known to posses this characterstic {Gillespie et al. 1992). The spedes is also capable of stimulating human gingival fibroblasts to produce IL6 and IL8. The role of C rectus has been somewhat difficult to determine because of the presence in plaque samples of a number of very closely related organisms such as Campylobactershowae and wolinella.

Eikenella corrodens

E. corrodens is a Gram-negative, capnophilic, asaccharolytic, regular, small rod with blunt ends. It has been recognized as a pathogen in other forms of disease, particularly osteomyelitis, infections of the central nervous system and root canal infections. This species was found more frequently in sites of periodontal destruction as compared with healthy sites. In addition, E. corrodens was found more frequently and in higher levels in active sites and in sites of subjects who responded poorly to periodontal therapy successfully treated sites harbored lower proportions of this species. E. corrodens has also been found in association with A. actinomyceterncomitans in some lesions of LJP. In tissue culture systems, E. corrodens has been shown to stimulate the production of matrix metallo-proteinases and IL-6 and IL-8 (Yumoto et al. 1999). While there is some association of this species with periodontal disease, to date it has not been particularly strong.

PEPTOSTREPTOCOCUUS MICROS

P.micros is a gram positive, anaerobic, small asaccharolytic coccus. It has long been associated with mixed anaerobic infections in the oral cavity and other parts of the body (Finegold 1977). Two genotypes can be distinguished with the smooth genotype being more frequently associated with periodontitis lesions than the rough genotype (Kremef etal.2000). P. micros has been detected more frequently and in higher numbers

at sites of periodontal destruction as compared with gingivitis or healthy sites and was elevated in actively breaking down sites The levels and frequency of detection of the species were decreased at successfully treated periodontal sites (Haffajee et al 1988). Studies of systemic antibody responses to suspected periodontal pathogens indicated that subjects with severe generalized periodontitis had elevated antibody levels to this species when compared with healthy subjects or subjects with LJP In a mouse skin model system, if was shown that P. micros in Combination with either P.intennedia or P.nigrescens could produce transmissible abscess

Selenomonas species

This species have been observed in plaque samples using light microscopy for many decades, the organisms may be recognized by their curved shape tumbling motility by the presence of a tuft of flagella inserted in concave side and, in good preparations. The Selenomonas spp. are Gram-negative curved, saccharolytic rods. The organisms have been somewhat difficult to grow and speciate.Moore et al. (1987) described six genetically and phenotypically distinct groups isolated from the human oral cavity. Selenomonas noxia was found at a higher proportion of shallow sites (PD < 4 mm) in chronic periodontitis subjects compared with similar sites in periodontally healthy subjects Further. S. noxia was found to be associated with sites that converted from periodontal health to disease.

EUBACTERIUM SPECIES

Certain Eubacterium species have been suggested as possible periodontal pathogens due to their increased levels in disease sites, particularly those of severe periodontitis E. nodarum. Eubacterium brachy and Eubacterium timidum are Gram-positive, strictly anaerobic, small, somewhat pleomorphic rods. They are often difficult to cultivate, particularly on primary isolation, and appear to grow better in roll tubes than on blood agar plates. Some of these species elicited elevated antibody responses m subjects with different forms of destructive periodontitis. The Eubacterium species appear to be promising candidates as periodontal pathogens; however, difficulty in their cultivation has slowed assessment of their contribution. It seems likely that the role of the Eubacterium species will be clarified when non-cultural methods are routinely

employed for their detection

THE "MILLERI" STREPTOCOCCI

Streptococci were frequently implicated as possible etiologic agents of destructive periodontal diseases in the early part of the last century. Cultural studies of the last two decades have also suggested the possibility that some of the streptococcal species are associated with and may contribute to disease progression. At this time, evidence suggests that the "milleri" streptococci. Streptococcus anginosus, S. constellates and S. intermedius might contribute to disease progression in subsets of periodontal patients. The species was found to be elevated at sites which demonstrated recent disease progression. Walker and co-workers (1993) found S. intermedius to be elevated in subsets of patients with refractory disease at periodontal sites which exhibited disease progression. Colombo ct al (1998) found that subjects exhibiting a poor response to SRP and then to periodontal surgery with systematically administered tetracycline had higher levels and proportions of S.constellatus. than subjects who responded well to periodontal therapy.

OTHER SPECIES

Obviously all periodontal pathogens have not yet been identified interest has grown in groups of species not commonly found in subgingival plaque as initiators or possibly contributors to the pathogenesis of periodonta! disease, particularly in individuals who have responded poorly to periodontal therapy. Species not commonly thought to be present in subgingival plaque can be found in a proportion of such subjects or even in subjects who have not received periodontal treatment. Emphasis has been placed on enteric organisms, staphylococcal species as well as other unusual mouth inhabitants. Slots et al examined plaque samples from over 3000 chronic periodontitis patients and found that 14% of these patients harbored enteric rods and pseudomonas. Enterobacter cloaceae, K.. pneumoniae, Pseudomonas aeruginosa, Klebsiella oxytoca and Enterobacter agglomicrans comprised more than 50% of the strains isolated. This group of investigators also examined 24 subjects with periodontal disease in the Dominican Republic and found that the prevalence of enteric rods in these subjects was higher than

levels found in subjects in the US. In the 16 of 24 subjects in which this group of organisms was detected, they averaged 23% of the cultivable microbiota. Rams et al. identified a number of species of staphylococci and enterococci in subjects with various forms of periodontal disease. The presence of unusual species in periodontal lesions suggests the possibility that they may play a role in the etiology of periodontal diseases. However, such roles must be evaluated in the same manner as the species discussed earlier in this section. It is worth noting that systemically administered ciprofloxacin improved the treatment response of patients whose periodontal pockets were heavily infected with enteric rods.

More recently, viruses including cytomegalo, Epstein Barr, papilloma and herpes simplex have been proposed to play a role in the etiology of periodontai diseases, possibly by changing the host response to the local subgingival microbiota. Human cytomegalo, Epstein Barr and herpes simplex virus were found more frequently in deteriorating periodontal sites than in control, stable periodontitis sites in the same subject.

Mixed infections

To this point, attention has been paid to the possible role of individual species as risk factors for destructive periodontal diseases. However, microbial complexes colonizing the subgingival area can provide a spectrum of relationships with the host, ranging from beneficial — the organisms prevent disease/to harmful - the organisms cause disease. At the pathogenic end of spectrum, it is conceivable that different relationships exist between pathogens. The presence of two pathogens at a site could have no effect or diminish the potential pathogenicity of one or other of the species. Alternatively, pathogenicity could be enhanced either in an additive or synergistic fashion. It seems likely that mixed infections occur in subgingival sites since so many diverse species inhabit this habitat. Evidence to support this concept has been derived mainly from studies in animals in which it was shown that combinations of species were capable of inducing experimental abscesses, even though the components of the mixtures could not. It is not clear whether the combinations suggested in the experimental abscess studies are pertinent to human periodontal diseases.

INTRA-ORAL HABITATS FOR PATHOGENIC SPECIES

Within hours after birth the sterile oral cavity will be colonized by low numbers of mainly facultative and aerobic bacteria (Socransky and Manganiello, 1971). From the second day on, anaerobic bacteria can be detected in the infant's edentulous mouth (Rotimi and Duerden, 1981; Evaldson *et al.,* 1982). Whether these organisms are already part of the developing indigenous flora, or just transient, is not known. After tooth eruption a more complex oral flora becomes established. For the oral microbiota, up to 500 different species have been described (Moore and Moore, 1994).

The bacteria that normally reside in the oral cavity *(i.e.* the indigenous microbiota) can select from different ecosystems for their habitat. On the basis of physical and morphological criteria the oral cavity can be divided into five major ecosystems (also called niches), each with distinct ecological determinants, *viz.* the buccal epithelium, the dorsum of the tongue, the supragingival tooth surface, the periodontal pocket (with its crevicular fluid, the root cementum and the pocket epithelium) and the tonsils. Most species (with the exception of spirochetes) are able to colonize all of them. Even in the edentulous mouth of infants or of denture wearers the proportions of periodontopathogens, with the exception of *A. actinomycetemcomitans* and *P. gingivalis* (Kononen *et al.,* 1992; Danser, 1996) can become very high. Thus the role of teeth as "port d' entree" for these bacteria seems negligible. This hypothesis is supported by the observation that a successful periodontal therapy (including surgical pocket elimination) had only a limited effect on the detection frequency of pathogenic species on the buccal mucosa and/or in the saliva (Danser *et al.*, 1996).

The intra-oral distribution of cariogenic species, on the contrary, seems relatively restricted to solid surfaces. For that reason *S. mutatis* is often called an

obligate periphyte (Tappuni and Challacombe, 1993). This tropism is supported by observations of Carlsson *et al.* (1969, 1970) who studied the life history of infection by mutans streptococci. In infants they could only recover these species from the time that the deciduous teeth erupted (Carlsson *et al.,* 1970). In a longitudinal observation of adults with severe dental caries, the cariogenic species fell below detection level after full-mouth extraction but reappeared in a few days after artificial denture insertion (Carlsson *et al.,* 1969). If these patients withdrew their dentures for a few days, the bacteria disappeared again. Based on these reports and on their own observations Caufield and Gibbons (1979) assumed that most of the *S. mutans* cells in the saliva or on the tongue are derived from the biofilm present on the teeth and that the mucosae could not act as a reservoir for the infection of teeth.

THE INTRA-ORAL TRANSLOCATION OF BACTERIA

During the last decade several publications reported the existence of intra-familial transmission of both cariogenic and periodontopathogenic species. The "intra-oral" translocation of these species, however, did not get much attention. This is surprising since most periodontopathic species can colonize different intra-oral niches. Transmission of these bacteria from one *locus* to another would jeopardize the outcome of periodontal therapy.

The importance of such intra-oral translocation is difficult to prove and/or quantify. It is nearly impossible to create a new sterile subgingival environment in an oral cavity with an established biofilm. However, the introduction of oral implants, especially of the 2-phase type, provided a new experimental set-up. Indeed, when the transmucosal part of the implant (the abutment) is inserted on top of the osseointegrated endosseous part, a new "virgin" surface is created. Since such implant-abutments can be replaced without any discomfort for the patient these "artificial" surfaces offer an excellent model to study the intra-oral translocation of bacteria (Quirynen *et al.,* 1996). These abutments are also useful for the study of the influence of surface characteristics, *(e.g.* roughness, surface free energy) on the initial supra and subgingival colonization (Quirynen *et al.,* 1994).

The transmission of bacteria to newly installed abutments was examined in a large group of partially edentulous patients by means of differential phase contrast micro-

scopy (Quirynen *et al.,* 1996), emphasizing the relative proportion of spirochetes. Since spirochetes almost exclusively colonize periodontal pockets and because they have been associated with periodontal breakdown (Listgarten and Helden, 1978), they were considered as ideal markers to study the intra-oral transmission from the teeth to the implant abutments. A few weeks after insertion, the proportion of spirochetes around the initially sterile abutments was comparable to that around the teeth, if the implants and the teeth were present in the same jaw. This observation not only suggested intra-oral translocation of bacteria between both abutment types, but also indicates that transmission preferentially occurs within a jaw (Quirynen *et al.,* 1996). Based on DNA-probes, Papaioannou *et al.* (1996a) reported a nearly comparable composition of the subgingival microbiota *(P. gingivalis. P. intermedia, B. forsythus)* around teeth and implants from partially edentulous patients with different types of periodontal infections. The similarity became more evident when the artificial abutments were located in deeper pockets, *i.e.* an environment closer to diseased sites in the natural dentition.

VEHICLES FOR THE INTRA-ORAL TRANSLOCATION OF PATHOGENIC SPECIES

The vehicle for the intra-oral translocation of pathogenic species is still an open question. Since all species survive in the saliva, this medium probably plays an important role. However, the intra-oral spread of *S. mutatis* by diffusion in the saliva was found to occur very slowly.

When, for example, streptomycin-resistant strains of *S. mutatis* were implanted in specific approximal regions (Edman *et al.,* 1975) or in specific artificial fissures (Svanberg and Loesche, 1978) of patients, the recovery of the organism after 26 weeks was limited to the immediate area of implantation, with very little transfer to distant tooth surfaces.

The translocation of periodontopathogens into a periodontal pocket by salivary flow is arguable, since the continuous outflow of crevicular fluid from the pocket (0.5 to 2.4mld~') makes the spontaneous entrance of saliva nearly impossible. Oral hygiene aids (which can penetrate the pocket up to 3 mm) or dental instruments (especially pocket probes and curettes) however, could contribute to this transmission.

ORAL HYGIENE AIDS

Several studies reported that daily used toothbrushes harbor a complex microbiota including periodontopathogens (Miiller *et al.,* 1989), cocci, haemophilus species and fungi (Malmberg *et al.,* 1994), and *S. mutans* (Svanberg, 1978). Most of these bacteria survive for a long period. Miiller *et al.* (1989), for example, examined toothbrushes from juvenile periodontitis patients infected by *A. actinomycetemcomitans* and found that 69% and 23% of the brushes harbored this species immediately after brushing and after 24h, respectively. Gizani *et al.* (1997) examined single used toothbrushes from young children with poly-caries and found high numbers of *S. mutans* and *Lactobaccillus* spp. up to 48 h after use. Glass and Jensen (1988) were able to recover considerable numbers of vital *Herpes simplex Vtrus-l* from toothbrushes up to 7 d after use. These authors concluded that it is conceivable that toothbrushes may contribute to a further dissemination of bacteria in the oral cavity, once a subject gets infected. Some authors suggest that such contaminated toothbrushes should be considered as a potential health risk, especially for immunosuppressed patients (Glass and Lare, 1986). The same applies to interdental aids (toothpicks, dental floss and interdental brushes).

DENTAL INSTRUMENTS

The possibility for iatrogenic intra-oral transmission of pathogenic species should be kept in mind. Indeed, dental explorers were found to harbor considerable amounts of *S. mutans* (Loesche *et al.,* 1973). The same research group showed that a "single" course of fissure probing (to explore caries) was sufficient to transmit streptomycine-resistant strains of *S. mutans* from an infected site to a non-infected fissure, even if this fissure already harbored an established microbiota (Loesche *et al.,* 1979).

Pocket probes are also often loaded with pathogenic species. Barnett *et al.* (1982) examined these instruments after probing a single deep pocket (> 6 mm) using light and electron microscopy. They found that all specimens contained large numbers of bacteria of which most had been morphologically associated with a pathogenic flora. Specifically, Gram-negative cocci, filaments and rods, flagellated filaments, and small and medium spirochetes were found. Christersson *et al.* (1985) reported that a "single" course of probing of an infected lesion from localized juvenile periodontitis patients resulted in a contamination of the instrument with up to 10^5 *A. actinomycetemcomitans* cells. Papaioannou *et al.* (1996b) evaluated the retrievabil-

ity of periodontopathogens with a pocket probe and compared it with paper points, the so called "golden standard" for plaque sampling. In general, the metal probes collected 1 log score less bacteria than the paperpoints, but the detection frequency for specific pathogenic species *(e.g. A. actinomycetemcomitans, P. gingivalis, P. intermedia* and *Campylobacter rectus)* was nearly identical.

CLINICAL RELEVANCE OF INTRA-ORAL TRANSLOCATION

SURVIVAL AFTER TRANSMISSION

Although dental and periodontal instruments and oral hygiene aids are contaminated with pathogenic organisms it is not yet clear whether these species can survive after transmission to a recipient site with its own ecosystem and established flora.

Several studies in cariology have shown that considerable quantities of 5. *mutans* can be transmitted with a dental explorer from one tooth to another and that the transmitted bacteria can survive for several days in their new environment (Svanberg and Loesche, 1978; Loesche *et al.,* 1979).

The transmission of periopathogenic species to non-infected periodontal pockets has also been documented. Christersson *et a!.* (1985) were able to successfully inoculate previously non-infected pockets with *A. actinomycetemcomitans* in localized juvenile periodontitis patients by a single course of probing. Twenty-eight out of the 30 healthy sites (previously negative for this bacterium) sampled immediately after "inoculation" yielded on average 16 x 10^3 *A. actinomycetemcomitans* cells *(versus* 10^s on the probe). One week later 8 of the 9 sites which were forcefully probed remained positive for *A. actinomycetemcomitans,* but at the end of the second week this number had reduced to 1 out of 9. Although the inoculation was only temporary, the question remained whether the inoculation could not become permanent if the site offered more suitable growth conditions *(e.g.* a deep pocket, as frequently encountered immediately after initial periodontal therapy).

Even syringe tips, used for repeated subgingival application of antibiotics, were found to become culture positive for antibiotic resistant bacteria (Preus *et al.,* 1993).

It was suggested to clean these tips, with paper tissue soaked in 70% ethanol before their repeated use, to prevent transmission of these resistant strains. The authors even speculated that such transmission was the explanation for some local, atypical gingival inflammations which were observed in their earlier clinical trials when the syringe tip was not cleaned between consecutive applications (Preus *el al.,* 1993).

FULL-MOUTH DISINFECTION AS TREATMENT

Periodontitis is classically treated by staged scaling and root planing of one quadrant or sextant per visit. The treatment of 1 quadrant normally takes 1 h or more. Intervals of 1 to 2 weeks are often applied. If the intra-oral translocation of pathogenic species occurs rapidly and to a large extent, it is conceivable that between treatments transmission of pathogens may occur from the untreated pockets or niches to the already treated quadrant(s). Since the recently treated sites posses the ideal environment for the growth of pathogenic species, such transmission might result in permanent re-inoculation.

This hypothesis was strongly supported by the results of a new treatment strategy, called "the full-mouth disinfection" (Quirynen *et al.,* 1995). In order to reduce the chances of recontamination, the new treatment scheme attempted to eradicate or at least suppress all periodontopathogens within a 24 h period. Moreover, the treatment did not only address the pockets but also other intra-oral niches. The full-mouth disinfection concept consisted of a combination of the following therapeutic efforts: a full-mouth scaling and rootplaning (the entire dentition in 2 visits within 24 h) to reduce the number of subgingival pathogenic organisms (Mousques *et al.,* 1980; Loos *et al.,* 1988); an additional subgingival irrigation (repeated 3 times within 10 min) of all pockets with *1%* chlorhexidine gel in order to kill remaining bacteria (Oosterwaal *et al.,* 1991); tongue brushing with 1% chlorhexidine gel for 1 min to suppress the bacteria in this niche; mouthrinsing with 0.2% chlorhexidine solution for 2 min to reduce the bacteria in the saliva (Rindom-Schi0tt *et al.,* 1976) and on the tonsils (the latter by gargling). Furthermore, re-colonization of the pockets was retarded by optimal oral hygiene, supported (for the first 2 months) by mouthrinsing with 0.2% chlorhexidine (Magnusson *et al.,* 1984). A pilot study, in which this approach was

compared to the above mentioned standard treatment (Quirynen *et al.,* 1995), showed that full-mouth disinfection resulted in an additional improvement. Clinically, the new approach resulted in a significant additional pocket reduction (5 1.5 mm) and a gain in attachment (1 mm) up to 8 months after therapy (Vandekerkhove *et al.,* 1996). From a microbiological point of view, at least for the first 2 months, a significantly larger reduction in the proportions of spirochetes, motile rods, and of several pathogenic organisms (A. *actinomycetemcomitans, P. gingivalis, P. intermedia, B. forsythus, E. corrodetis, F. nucleatum, Peptostreptococcus micros, C. rectus,* and *Eubaderium* spp.) was observed. The proportion of "beneficial" bacteria increased significantly more in the group of patients treated with full-mouth disinfection when compared to those treated in the standard way (Bollen *et al.,* 1996).

The beneficial effect of the full-mouth approach is supported by the microbiological observations in two recent studies. Tonetti *et al.* (J995) for example compared the microbial changes when tetracycline fibers (local application of antibiotics) were only applied to the two deepest pockets in the mouth with those found when all sites with a depth > 3 mm were treated. After 6 months, significant additional improvements (clinical as well as microbiological) were recorded in the group of patients with the more complete approach. A study by Nowzari *et al.* (1996) compared the amount of guided tissue regereration and membrane contamination in a single study site between patients with and without previous periodontal therapy in the remaining dentition. The group of patients with no pockets 5mm (besides the study site) showed significantly less membrane contamination and significantly more clinical gain in attachment.

CONCLUSION :-

With continuous improvement and advances the field of microbiology and immunology the exact role played by the periodontal pathogens in periodontal diseases is being elucidated. Progress in delineating the molecular basis of periodontal pathogenesis in coming years will be greatly increased due to tremendous advances in DNA sequence information and techniques for its use in molecular analysis.