Rev. Port. de Est. e Cir. Maxilofac.
31 (2) 91-96 1990

ACTINOBACILLUS ACTINOMYCETEMCOMITANS AND BLACK-PIGMENTED BACTEROIDES IN ADVANCED PERIODONTITIS IN MAN. THEORETICAL AND PRACTICAL CONSIDERATIONS.

Gil A.P. Alcoforado[1] and Jorgen Slots[2]

RESUMO: Neste artigo, após uma breve referência à microflora existente no sulco gengival saudável, discutem-se vários aspectos do comprometimento etiológico do *A. actinomycetemcomitans*, do *B. gingivalis* e do *B. intermedius* como agentes etiológicos das várias formas de doenças periodontais. Para além da enumeração de algumas das suas características próprias, é dada especial atenção a determinados factores de virulência que podem estar estrictamente relacionados com o desenvolvimento da doença periodontal.

Termina-se, apresentando-se a racionalização do uso de antibióticos para o tratamento dos vários tipos de doenças periodontais, quando devidamente suportado por colheita e análise microbiológica prévia e respectivo antibiograma.

Palavras-chave: Doença Periodontal, Actinobacillus Actinomycetemcomitans, Bacteroides SPP.

INTRODUCTION

Much progress has been made in understanding the microorganisms involved in destructive periodontal diseases, the pathogenic potencial of major periodontopathic bacteria, and means of eliminating or markedly reducing pathogens in severe periodontal infections.

In 1964, Harald Loe and co-workers (4) convincingly demonstrated that the formation of dental plaque led to the development of gingivitis. The study was a major breakthrough for the time. It deeply influenced the major concepts of the etiology of gingivitis and it also formed the rationale for treating inflammatory periodontal disease. However, the "experimental gingivitis" studies by Loe did not address the question of destructive periodontal disease. The non-specific microbiological hypothesis which was partly derived from the experimental gingivitis model did not explain why destructive periodontal disease generally occurs in selected sites of a given dentition. It also could not explain why some patients did not show a good correlation between degree of gingivitis and severity of periodontitis. These clinical observations gave rise to the thought that specific pathogenic bacteria were associated with destructive periodontitis.

[1]Department of Periodontics,
Escola Superior de Medicina Dentária
Lisboa, Portugal
[2]Department of Periodontics,
School of Dental Medicine
University of Pennsylvania
Philadelphia. PA, USA

Recebido para publicação em 19/3/90

To date, more than 300 different bacterial species have been isolated from the periodontal pocket. It is unlikely that all those different microorganisms are equally important pathogens in periodontitis. In fact, recent research indicates that relatively few bacteria are involved in progressing periodontitis. Major suspected periodontopathogens include *Bacteroides gingivalis, Bacteroides intermedius, Actinobacillus actinomycetemcomitans* and possibly *Eikenella corrodens, Wolinella* species, *Fusobacterium* species and various non-pigmented *Bacteroides* species. This paper will focus on the importance of *B. gingivalis, B. intermedius* and *A. actinomycetemcomitans* in severe periodontitis in man.

Periodontal microflora-overview

Healthy gingiva is associated with cocci and non-motile rods while adult periodontitis is characterized by motile rods and spirochetes, each of these morphotypes comprising about 25-30% of the total microflora. A marked shift in the subgingival microbial composition with disease development was first convincingly demonstrated in the experimental gingivitis studies in the mid 1960s (18). Culture studies in the mid 1970s on groups of patients with healthy periodontium, gingivitis, adult periodontitis and localized juvenile periodontitis further clarified the composition of the periodontopathic microflora (6).

A preponderance of gram-positive facultative organisms was found in the subgingival microflora of healthy periodontium. By contrast, various gram-negative anaerobic, motile and non-motile bacterial species predominated in deep periodontitis lesions. Thus, a markedly increased presence of gram-negative anaerobic bacteria is associated with the development of periodontitis. Is this dramatic change in microbial composition the cause for the disease or is the microbial shift merely secondary to the pathological changes?

Association of black-pigmented Bacteroides and A. actinomycetemcomitans with progressing periodontitis

Although cause and effect in periodontal microbiology has not yet been definitely established, various lines of evidence link *B. gingivalis, B. intermedius* and *A. actinomycetemcomitans* to progressive periodontitis. *B. gingivalis* and *B. intermedius* comprise black-pigmented non-motile gram-negative anaerobic rods and *A. actinomycetemcomitans* is a gram-negative non-motile capnophilic short rod.

Two studies have associated these 3 bacteria with advancing periodontitis in adults. Tanner et al. (15) demonstrated high proportions of one or more of these organisms in most advancing periodontitis lesions and a much lower occurrence in periodontal sites in remission. Slots et al. (11) also detected, in a large patient samples, a significantly higher prevalence and higher proportions of these bacteria in progressing than in non-progressing periodontal sites.

A. actinomycetemcomitans is considered a pathogen of major importance in localized juvenile periodontitis. In the USA where this disease occurs in less than 1% of the population, *A. actinomycetemcomitans* has been reported to occur in 90% or more of localized juvenile periodontitis patients but in less than 20% of healthy or minimally diseased individuals and then only in very low numbers. (13,5,20).

In Panama, however, where 5-10% of a selected Black population exhibits localized juvenile periodontitis, *A. actinomycetemcomitans* was also found in 60% of normal young individuals (3). On the basis of these data, it is reasonable to speculate that if the general population demonstrates a high level of infection by a periodontopathogen, the risk is greater that susceptible individuals will acquire the organism and get the disease.

The epidemiology of *A. actinomycetemcomitans* was studied by Zambon et al. (20). In five families investigated, some family members exhibited localized juvenile periodontitis, some others severe periodontitis, and still others minimal periodontal disease. *A. actinomycetemcomitans* was present in many of the periodontitis patients but was generally not recovered from the healthy individuals. A significant finding was that each infected subject within a family harbored the same *A. actinomycetemcomitans* biotype and serotype. Since the *A. actinomycetemcomitans* species comprises ten biotypes and three serotype, this intrafamilial distribution of *A. actinomycetemcomitans* is most unlikely to occur by chance. This data suggests that *A. actinomycetemcomitans* spreads from family member to family member. It

may be that the familial prevalence of localized juvenile periodontitis is partly the result of intrafamilial bacterial spread. If so, the rationale exists for treating whole family units in order to avoid transmission and reinfection between family members.

Bacterial antibodies may also serve to incriminate an organism in the etiology and pathogenesis of periodontal disease. Two recent studies found significantly elevated serum IgG antibody levels against *B. gingivalis, B. intermedius* and *A. actinomycetemcomitans* in periodontitis patients compared to healthy controls (16,19). Tew et al. (17) also showed that *B. gingivalis* and *A. actinomycetemcomitans* were the only two organisms of nine oral bacterial species tested to exhibit markedly higher antibody levels in gingival crevicce fluid than in serum. This elevated gingival crevice fluid antibody level is suggestive of local antibody production and further underscores the association of these two bacteria with advanced periodontitis.

Bacterial virulence factors

B. gingivalis and *B. intermedius* possess a number of pathogenic properties which make them particularly well-equipped to overcome protective host defenses (9). One key mammalian cell in the periodontal defense is the polymorphonuclear leukocyte. The protective capability of polymorphonuclear leukocytes may be severely impaired by *B. gingivalis* and *B. intermedius*. These bacteria have the capacity to inhibit neutrophil chemotaxis and they possess capsules with antiphagocytic properties. Some strains of these organisms also elaborate proteases with the potencial to degrade opsonizing immunoglobulins and complement proteins. Even if neutrophil chemotaxis and phagocytosis did take place, the organisms still have the ability to overcome intraphagocytic killing mechanisms. However, the infectious disease process is considerably more complex. The host will attempt to neutralize the bacterial aggressins by antibody production. These antibodies, on the other hand, may be degraded by the bacterial immunoglobulin proteases. These enzymes, in turn, may act as antigens and become neutralized by specific antibodies. *B. gingivalis* also elaborates enzymes with potential to degrade plasma proteinase inhibitors and collagen. These enzymes may also act as antigens and give rise to neutralizing antibodies. If destructive enzymes are the dominating determinant, a breakdown of the periodontal connective tissue attachment may take place. If antibodies are in relative surplus (which is probably most frequently the case), the disease may be in a state of remission. The dynamic interplay between bacterial proteolytic enzymes and antibodies may exemplify how the host-parasite interrelationship determines the disease status of the periodontium.

A. actinomycecomitans produces a large number of cytotoxic factors (14). It possesses leukotoxic activity against human blood and gingival crevice polymorphonuclear leukocytes and human blood monocytes; it elaborates a lymphocyte suppressive factor; it produces a fibroblast suppressive factor; it releases an epithelial cell inhibitory factor; it liberates a suppressive factor inhibiting human endothelial cells; and it demonstrates a highly potent lipopolysacharide which is capable of stimulating in vitro bone resorption and induce classical endotoxin responses. The high virulence capacity of *A. actinomycetemcomitans* enables this organism to invade and colonize the gingival connective tissues (9). However, similarly to black-pigmented *Bacteroides* periodontitis, *A. actinomycetemcomitans* periodontitis is associated with high levels of serum and gingival crevice fluid antibodies against the leukotoxin and other of the organism's noxious products. Again, a delicate balance between *A. actinomycetemcomitans* virulence factors and various protective immune defense systems most probably determines the rate and extent of the periodontal destruction.

The host-parasite relationship may become increasingly more complex when more than one pathogen is interacting. As an example, the leukotoxin of *A. actinomtcetemcomitans* may lyse gingival and periodontal pocket polymorphonuclear leukocytes. This may release lysosomal enzymes including collagenase which may destroy periodontal collagen fibers. *B. gingivalis* also produces a collagenase which may potentiate this type of destruction. However, the proteinaceous leukotoxin of *A. actinomycetemcomitans* in addition to be neutralized by specific antibodies may also be destroyed by proteolytic enzymes of *B. gingivalis*. In that event, the proteases of *B. gingivalis* may serve a protective function. Nevertheless, these same enzymes also act as antigens, giving rise to antibody production. As can be seen from Fig. 1,

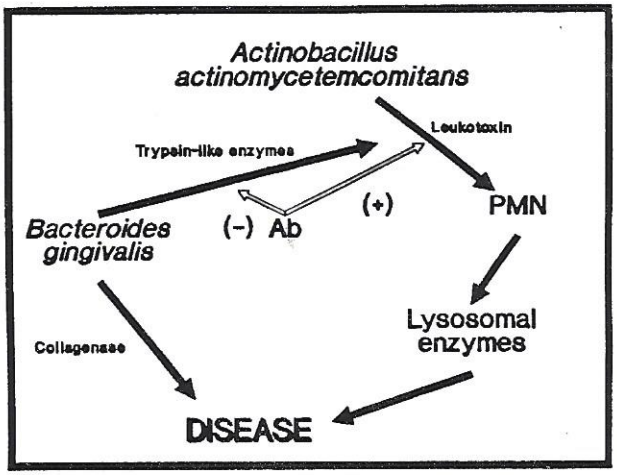

Fig. 1

antibodies may then exert opposite biological effects. While the antileukotoxin antibody is a protective one, anti-*B. gingivalis* protease antibody by preventing destruction of the leukotoxin may contribute to disease progression. If such interactions indeed take place in periodontal disease is a matter for further study.

Periodontal therapy

Knowledge of the specific infectious nature of advancing periodontitis can help considerably in planning periodontal therapy. Bragd et al. (2) investigated 10 patients, 31 to 59 years of age, who revealed continuous loss of alveolar bone and probing periodontal attachment level despite comprehensive surgical periodontal therapy. The initial therapy had included patient motivation, instruction in oral hygiene techniques, removal of plaque retention factors, as well as supra- and subgingival scaling and root planing. Tooth surfaces which bled on probing and with periodontal pocket depth exceeding 4 mm after the initial phase had been subjected to periodontal surgery using a modified Widman flap technique or, in some cases, gingivectomy. Professional tooth cleaning had been carried out every 3 months for a prolonged period of time. After therapy, none of the patients showed supragingival plaque on more than 15% of all tooth surfaces. These patients had received a total of 50 to 100 hours of active periodontal therapy. *A. actinomtcetemcomitans* occurred in 81% of deep progressive periodontitis lesion studies (that is 21 out of 26 sites), in a mean proportion of 8% of the cultivable microflora (2). In comparison, this organism was only found in 5% of deep non-progressive sites and averaged less than 1% of the total isolates (11). Five progressive lesions which did not yield *A. actinomycetemcomitans* showed high proportions of *B. gingivalis* or *B. intermedius.* These data implicating *A. actinomycetemcomitans* as a major organism in refractory adult periodontitis agree with recent microbiological findings on recurrent adult periodontitis by Carlsson and Sundqvist, Umea, Sweden (personal communication). One reason for the failure of the mechanical debridement to resolve the periodontal *A. actinomycetemcomitans* infections may be the ability of the organism to invade subepithelial periodontal tissues and thereby partly escape the cleansing effects of periodontal instruments. If so, the rationale exists for using systemic antibiotic therapy to eliminate pathogenic organisms from the gingival tissues.

Four refractory adult periodontitis patients with substantial numbers of subgingival *A. actinomycetemcomitans* were treated with systemic tetracycline (1g tetracycline — HC1/day/21 days). All 9 test periodontal pockets were free of detectable *A. actinomycetemcomitans* and *B. gingivalis* at 2 months after tetracycline therapy. Six periodontal pockets showed *B. intermedius* post treatment, but in numbers likely too low to sustain a destructive disease process. Clinically, the removal of the pathogenic microflora parallelled a decrease in probing pocket depth of 2 mm or more and a distinct gain of radiographic crestal alveolar bone.

These data and similar treatment findings obtained in localized juvenile periodontitis patients (10) show that an appropriate systemic antimicrobial therapy is a valuable adjuncy in treatment of certain cases of advanced periodontitis.

Microbiology in diagnostic periodontics

In order to institute a proper antibiotic therapy in periodontal disease, the specific organisms responsible for the disease and their antibiotic susceptibility pattern must be known. *B. gingivalis, B. intermedius* and *A. actinomycetemcomitans*,in addition to a few other subgingival bacteria, (8), are organisms whose presence in the periodontal

pocket should be assessed. A microbiological diagnosis can be performed by an oral microbiology service or by the practitioner utilizing simplified microbiological identification means (7). Furthermore, since strains of virtually each oral bacterial species may vary greatly in antimicrobial susceptibility (1), an in vitro susceptibility test should optimally be carried out for isolates of each individual patient.

We presently recommend the following protocol for treating advanced periodontal disease:

1. Conventional periodontal therapy including thorough periodontal scaling and root planing, preferentially in conjunction with periodontal surgery to gain acess to the affected root surfaces.
2. Monitoring of the clinical course and subgingival microflora.
3. Antimicrobial therapy in recurrent cases. Treatment with antimicrobial agents should be short-term and intensive aiming, optimally, at eradicating the pathogenic components of the subgingival microflora.
4. Microbiological control of treatment efficacy and patient's compliance.

Concluding remarks

There seems to be little doubt that systemic antibiotic therapy can provide a very valuable adjunct to conventional periodontal treatment. However, in order to institute an appropriate antibiotic therapy, certain guidelines must be adhered to. We should reemphasize the importance of monitoring the periodontal microbiota prior to and at the end of the active therapy, as well as during the maintenance care phase. This and an antibiotic susceptibility testing of the suspected pathogens should always be part of antibiotic treatment of periodontal diseases.

Patients' compliance is a potencial problem. Therefore, patients should be given precise information and strong motivation before the start of any antibiotic treatment.

It is hoped that a microbiological approach to periodontal disease management will substantially improve the efficiency and effectiveness of current periodontal treatments.

REFERÊNCES

1. **H. Baker, P.J., Evans, R.T., Slots, J. & Genco, R.J.** Antibiotic susceptability of anaerobic bacteria from the human oral cavity. Journal of Dental Research 1985, **64**:1233-1244.
2. **Bradg, L., Wikstrom, M. & Slots, J.** Clinical and microbiological study of "refractory" adult periodontitis. Journal of Dental Research 1985, **64**:234 (abstract no. 538).
3. **Eisenmann, A.C., Eisenmann, R., Sousa, O. & Slots, J.** Microbiological study of localized juvenile periodontitis in Panama. Journal of Periodontology 1983, **54**:712-713.
4. **Loe, H., Theilade, E. and Jensen, S.B.** Experimental gingivitis in man. Journal of Periodontology 1965, **36**:177-187.
5. **Mandell, R.L. & Socransky, S.S.** A selective medium for *Actinobacillus actinomycetemcomitans* and the incidence of the organism in juvenile periodontitis. Journal of Periodontology 1981, **52**:593-598.
6. **Slots, J.** Subgingival microflora and periodontal disease. Journal of Clinical Periodontology 1979, **6**:351-382.
7. **Slots, J.** Rapid identification of important periodontal microorganisms by cultivation. Oral Microbiology and Immunology 1986, **1**:48-55.
8. **Slots, J. & Dahlen, G.** Subgingival microorganisms and bacterial virulence factors in periodontitis. Scandinavian Journal of Dental research 1985, **93**:119-127.
9. **Slots, J. & Genco, R.J.** Black-pigmented *Bacteroides* psecies, *Capnocytophaga* species, and *Actinobacillus actinomycetemcomitans* in human periodontal disease: virulence factors in colonization, survical and tissue destruction. Journal of Dental Research 1984, **63**:412-421.
10. **Slots, J. & Rosling, B.G.** Suppression of the periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. Journal of Clinical Periodontology 1983, **10**:465-486.
11. **Slots, J., Bragd, L., Wikstrom, M. & Dahlen, G.** The occurrence of *Actinobacillus actinomycetemcomitans, Bacteroides gingivalis* and *Bacteroides intermedius* in destructive Periodontology 1986, **13**:570-577.
12. **Slots, J., Emrich, L.J., Genco, R.J. & Rosling, B.G.** Relationship between some subgingival bacteria and periodontal pocket depth and gain or loss of periodontal attachment after treatment of adult periodontitis. Journal of Clinical Periodontology 1985, **12**:540-552.
13. **Slots, J., Reynolds, H.S., Genco, R.J.** *Actinobacillus actinomycetemcomitans* in human periodontal disease: A cross-sectorial microbiological investigation. Infection and Immunity 1980, **29**:1013-1020.
14. **Taichman, N.S., Tsai, C.-C., Shenker, B.J. & Boehringer, H.** Neutrophil interactions With oral bacteria as a pathogenic mechanism in periodontal diseases. Advances in Inflammation Research (ed. G. Weissmann) 1984, **8**:113-142.
15. **Tanner, A.C.R., Haffer, C., Bratthall, G.T., Visconti, R.A. & Socransky, S.S.** A study of the bacteria associa-

ted with advancing periodontitisd in man. Journal of Clinical Periodontology 1979, 6:278-307

16. **Tew, J.G., Marshall, D.R., Moore, W.E.C., Best, A.M., Palcanis, K.G. & Ranney, R.R.** Serum antibody reactive with predominant organisms in the subgingival flora of young adults with generalized severe periodontitis. Infection and Immunuty 1985a, **48**:303-311.

17. **Tew, J.G., Marshall, D.R., Burmeister, J.A. & Ranney, R.R.** Relationship between gingival crevicular fluid and serum antibody titers in young adults with generalized and localized periodontitis. Infection and Immunity 1985, **49**:487-493.

18. **Theilade, E., Wright, W.H., Jensen, S.B. and Loe, H.** Experimental gingivitis in man. II. A Longitudinal Clinical and Bacteriological Investigation. Journal of Periodontal Research 1966, **1**:1-13.

19. **Tolo, K. & Schenck, K.** Activity of serum immunoglobulins G, A, and M to six anaerobic oral bacteria in diagnosis of periodontitis. Journal of Periodontal Research 1985, **20**:113-121.

20. **Zambon, J.J., Christersson, L.A. & Slots, J.** *Actinobacillus actinomycetemcomitans* in human periodontal disease: Prevalence in patient groups an distribution of biotypes and serotypes within families. Journal of periodontology 1983, **54**:707-711.