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Ecological considerations in the treatment of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* periodontal infections

SIGMUND S. SOCRANSKY, ANNE D. HAFFAJEE, LAURIE ANN XIMENEZ-FYVIE, MAGDA FERES & DONNA MAGER

Personal historical perspective

The appearance of this issue of PERIODONTOLOGY 2000 is a tribute to the sustained efforts of an international group of oral microbiologists, immunologists and clinical investigators. When one of us (SSS) began work in this field in 1957, *Actinobacillus actinomycetemcomitans* was unknown in periodontal microbiology and *Porphyromonas gingivalis* was not known as such, although the "black-pigmented *Bacteroides*" had achieved some notoriety in studies of experimental mixed infections. In the late 1950s, the importance of plaque accumulation in the pathogenesis of periodontal diseases was once more re-established. With a few exceptions, most investigators appeared to subscribe to the notion of the nonspecific plaque hypothesis in terms of the causation of periodontal diseases. Plaque composition was thought to be similar from subject to subject and site to site within a subject. Abundant plaque formation was thought to lead to gingivitis and gingivitis eventually to periodontitis. The only studies seeking specific causative agents of disease were studies of mixed infections in animal model systems. Early studies of mixed infections focused on a fusospirochetal complex (85, 86, 98), whereas later studies emphasized infections in which "*Bacteroides melaninogenicus*" was a key component (65, 66, 100). This last group of studies was a precursor to the introduction of *P. gingivalis* as a key "player" in periodontal infections.

The re-introduction of the concept of specificity in the causation of periodontal diseases gained impetus from the detection of a specific spirochete in lesions of acute necrotizing ulcerative gingivitis (59, 63) and the demonstration that transmission of periodontal disease in a hamster model system was due to infection by a specific species now known as *Actinomyces viscosus* (43, 44, 47, 48). The key to the recognition of the role of *A. actinomycetemcomitans* in periodontal diseases and the firm re-establishment of the "specific plaque" hypothesis came from cultural studies of the subgingival microbiota detected in a clinical condition known at that time as periodontosis (now known as localized juvenile periodontitis). Studies in Copenhagen and Boston carried out by Slots (95) and Newman et al. (72, 73) demonstrated that the microbiota recovered from lesions in these subjects differed from that observed in healthy periodontal sites in the same subjects. Notable were the high levels of several unidentified gram-negative rod species (*A. actinomycetemcomitans* was not recognized as such until 1979 (105)). These studies indicated that lesion sites harbored different species than healthy sites in the same subject but did not determine whether the microorganisms caused the lesions or whether the habitat of the microbiota selected the detected species. Therapy studies reviewed in Slots & Ting in this volume (97) demonstrated that control of the species in the lesion led to disease remission indicating unequivocally that the resident species caused the infection. The focus on *A. actinomycetemcomitans* as the key organism in this infection came about primarily from studies of the local and serum antibody response in subjects with localized juvenile peri-

odontitis (51). Details of the relationship of *A. actinomycetemcomitans* with localized juvenile periodontitis and other periodontal diseases can be found in another chapter in this volume (97).

The recognition of the importance of *P. gingivalis* in periodontal diseases followed a similar course although hints of its role could have been gleaned from studies of experimental mixed infections (65, 66, 100). Once more, the key studies were cultural. Slots (96) and Tanner et al. (105) sampled adult periodontitis lesions and found high proportions of gram-negative rod species that formed black colonies on anaerobically incubated blood agar plates. Samples from healthy sites in the same subjects or healthy subjects exhibited lower proportions or none of the species that formed black-pigmented colonies. These studies were rapidly confirmed and extended (34). Successful control of the species led to control of disease (97). Elevated antibody responses to these organisms were found in subjects with different forms of periodontitis when compared with serum samples from periodontally healthy subjects (51).

A. actinomycetemcomitans and *P. gingivalis*: current status

This volume of PERIODONTOLOGY 2000 has summarized much of the available data concerning the role of *A. actinomycetemcomitans* and *P. gingivalis* in human periodontal disease. The data provide compelling evidence that these species contribute to the initiation and/or progression of destructive forms of periodontitis, including localized juvenile periodontitis and adult forms of periodontitis (34). The data also indicate that elimination or suppression of these taxa improves the probability of long-term stability of the periodontal structures and the likelihood of maintaining a natural dentition (34, 97). Extensive reviews in this volume summarize the exceptionally wide array of potential virulence factors produced by the species and indicate possible mechanisms of pathogenicity (27, 38, 77). The emphasis on these species is entirely justified, even though it is recognized that other species can and do contribute to periodontal pathogenesis.

After reading this volume, the reader will become familiar with the depth and breadth of investigations involving *A. actinomycetemcomitans* and *P. gingivalis*. It is interesting that about 1000 articles have been published since January 1995 involving one or both of these species, an average of close to one paper per day. Yet studies of these species are about to undergo a quantum acceleration. Ongoing investigations initiated at Oklahoma University and Forsyth Dental Center will provide the DNA sequences of the entire genomes of both *A. actinomycetemcomitans* and *P. gingivalis* within 1 to 2 years. This information will accelerate studies by molecular biologists attempting to determine the genes and regulation of genes governing the metabolism and survival of the organisms as well as the genes that encode virulence factors affecting the host. Knowledge of the genetic makeup and regulation of the 2 species will provide new routes to the control of these taxa in the human oral cavity.

Future directions: the pivotal role of oral ecology

On a basic science level, future research on *A. actinomycetemcomitans* and *P. gingivalis* is extremely promising. Undoubtedly major advances will be made in our understanding of the mechanisms of pathogenicity and relative contributions of the species to clinical disease. Less certain is our ability to control infections by these taxa. We have made promising beginnings in defining therapeutic approaches as outlined in Slots & Ting (97); however, over time the beneficial effects of active periodontal therapy tend to diminish. This is manifest in the recognized need for an indefinite period of professional and self-performed maintenance. These recognitions suggest that the species under discussion re-emerge (or other species emerge), leading to the need for maintenance or additional therapy. This is an unsatisfactory state for the profession and for the patient.

When procedures to control oral organisms are examined, it is somewhat surprising that the procedures are as successful as they appear to be. Current procedures to control periodontal infection may be divided into mechanical and chemotherapeutic. When therapies such as scaling and root planing or periodontal surgery are performed, it is highly unlikely that every cell of *A. actinomycetemcomitans* or *P. gingivalis* can be removed. Yet improvement often occurs. Why? Similarly, the use of chemotherapeutic agents can have a profound effect on species in subgingival plaque. However, given the terrain colonized by the organisms, and the notorious difficulty that antibiotics have in affecting organisms in biofilms, it is unlikely that these agents can completely rid the periodontal pocket or the oral cavity of either *A. actinomycetemcomitans* and *P. gingivalis*. However, the agents are often helpful. What do they do?

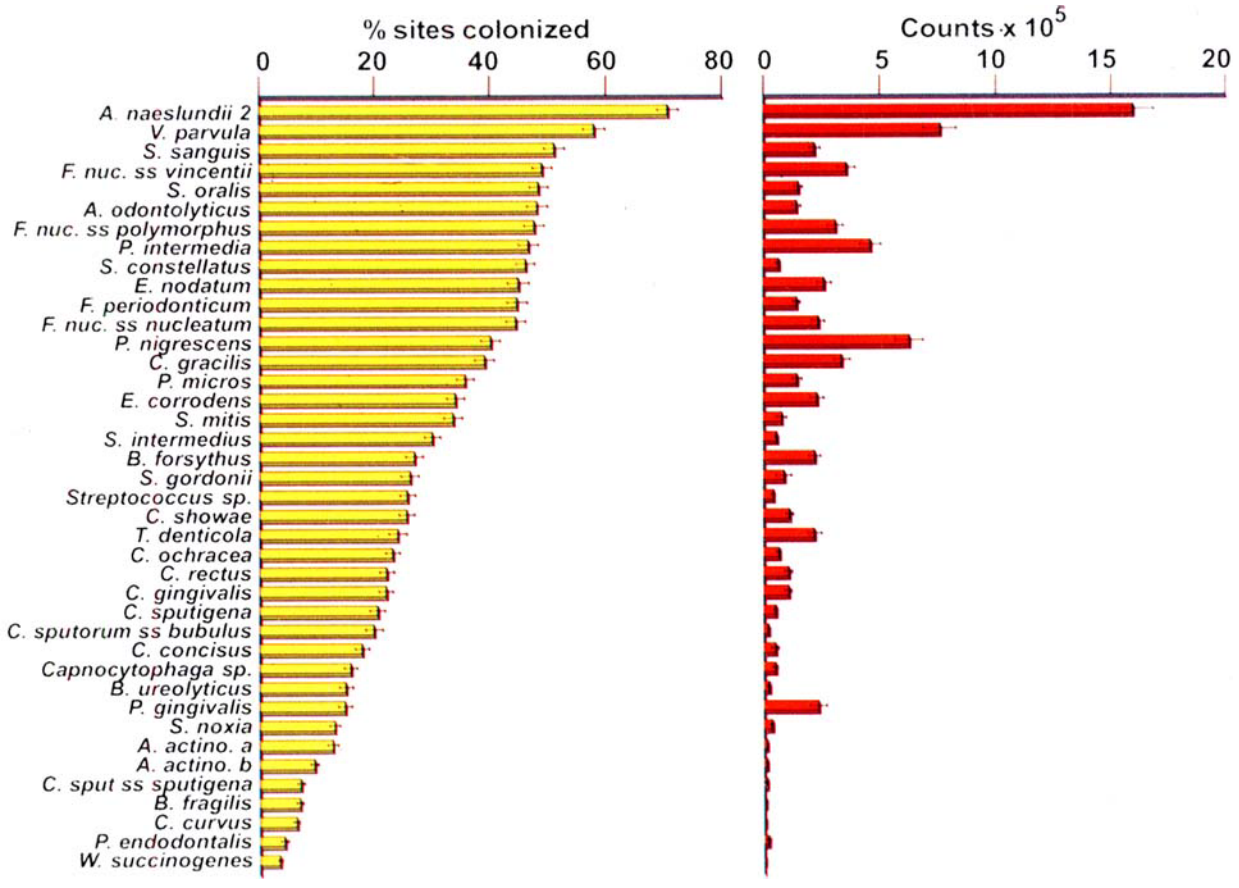

Fig. 1. Prevalence and levels of major taxa in human subgingival plaque samples. The left panel depicts the percentage of sites colonized (prevalence) by the indicated taxa and the right panel, mean counts $\times 10^5$. Subgingival plaque samples were taken from the mesial aspect of all teeth present in each of 213 adult subjects at one or more visits and evaluated individually for the presence and levels of 40 bacterial taxa using checkerboard DNA-DNA hybridization. The percentage of sites colonized were determined in each subject and averaged across subjects. Similarly counts of each species were determined at each site, averaged across sites within the subject and then across subjects. The total number of samples was 16,330.

It seems likely that both mechanical and chemotherapeutic approaches manifest their beneficial effects by affecting the ecological relationships of species within plaque and with the host. Other than by the development of a "magic bullet" (such as a vaccine or a completely species-specific antimicrobial agent), the most promising approach to the control of periodontal infections would appear to be by controlling their ecology. The first step would be to understand their ecological relationships with the host and other oral bacterial species resident in the oral cavity (for additional data on ecology of *A. actinomycetemcomitans* and *P. gingivalis* see Asikainen & Chen (8)). Therapies could then be designed to adjust the ecological relationships in order to diminish pathogen load and foster local environments or colonizing species that would keep pathogens consistently at low levels. In the next sections, the colonization patterns of *A. actinomycetemcomitans* and *P. gingivalis* will be examined as well as the factors that influence this colonization pattern: that is, the ecological relationships of the taxa. This will be followed by an examination of the effects of current therapeutic procedures on ecological relationships.

Prevalence and levels of *A. actinomycetemcomitans* and *P. gingivalis* in subgingival plaque and relationship to disease status

Numerous studies have revealed a relationship between *A. actinomycetemcomitans* and *P. gingivalis* and various forms of periodontal disease (34). How-

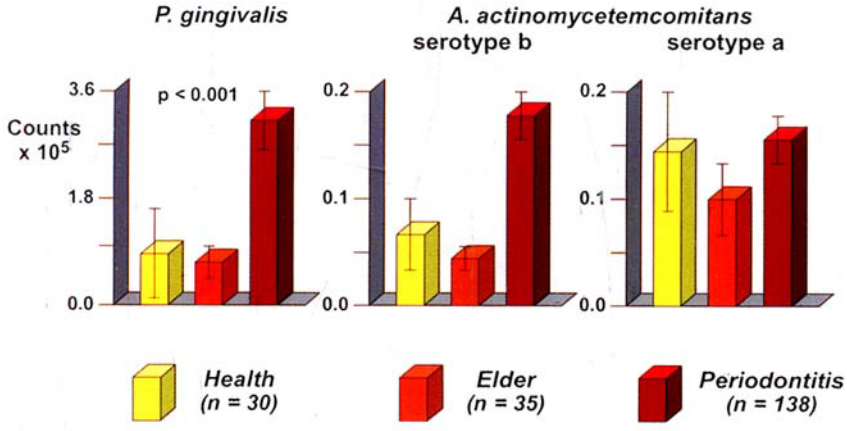

Fig. 2. Bar chart of the mean counts ($\times 10^5$) of *P. gingivalis* and *A. actinomycetemcomitans* serotypes a and b in periodontally healthy subjects (n=30), well-maintained elderly subjects (n=35) and periodontitis subjects (n=138). The mean counts for each of the three taxa were computed for a subject and then averaged across subjects in each group. Significance of differences among groups was evaluated using the Kruskal-Wallis test and adjusted for multiple comparisons (37). The vertical bars and whiskers represent the mean±SEM.

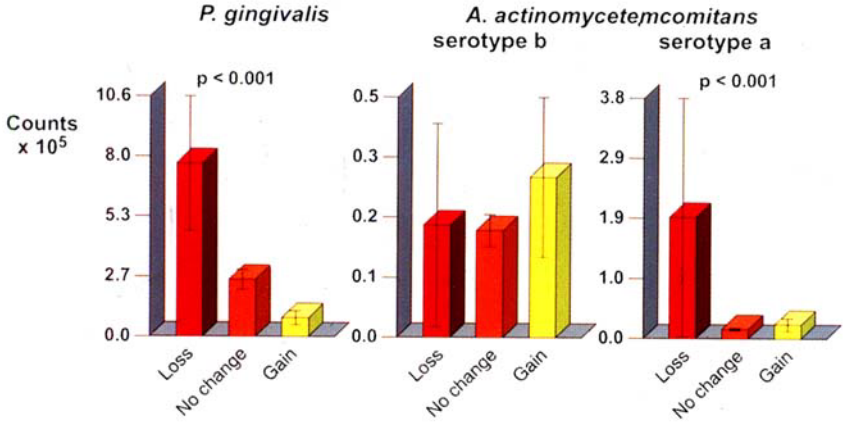

Fig. 3. Bar chart of the mean counts ($\times 10^5$) of *P. gingivalis* and *A. actinomycetemcomitans* serotypes a and b at sites that exhibited >2 mm attachment loss, gained >2 mm attachment or showed changes between these values in the 3 months prior to sampling. The mean counts for each of the 3 taxa in each attachment level change category were computed for a subject and then averaged across subjects for each category. The significance of differences among groups was evaluated using the Kruskal-Wallis test and adjusted for multiple comparisons. The vertical bars and whiskers represent the mean±SEM.

ever, the species are not particularly prevalent or at high numbers in subgingival plaque samples if a wide range of periodontal sites are examined including periodontally healthy sites in diseased subjects or sites in periodontally healthy subjects. Fig. 1 presents the prevalence and levels of major taxa in subgingival plaque samples obtained from adult subjects. The dominant species detected was *Actinomyces naeslundii* genospecies 2 (*A. viscosus*) both in terms of prevalence and level. Other prevalent species included *Veillonella parvula, Streptococcus sanguis, Fusobacterium nucleatum* subsp. *vincentii, Streptococcus oralis* and *Actinomyces odontolyticus.* The recognition that *A. actinomycetemcomitans* and *P. gingivalis* are much less frequently detected and in lower numbers than many other subgingival species in no way detracts from their likely role in pathogenesis of disease. Fig. 2 (adapted from Haffajee et al. (36)) presents the mean counts ($\times 10^5$) of *A. actinomycetemcomitans* and *P. gingivalis* in periodontally healthy subjects, well-maintained elderly subjects who had been successfully treated for periodontitis or subjects with adult periodontitis. *P. gingivalis* was significantly elevated in subjects with adult periodontitis even when the data were adjusted for 40 multiple comparisons as described in the original article (36). *A. actinomycetemcomitans* serotype b was significantly elevated in the periodontitis group if data were not adjusted for multiple comparisons. Fig. 1 and 2 indicate that *A. actinomycetemcomitans* and *P. gingivalis* are not common or in high numbers in most human subjects; however, they are at higher levels (and more prevalent, data not shown) in subjects with periodontitis. The taxa also relate to recent disease progression at individual sites. Fig. 3 presents the mean levels of the species at sites that exhibited >2 mm attachment loss, >2 mm attachment "gain" or changes between these values in the 3 months prior to sampling in 138 adult periodontitis subjects. The mean counts of both *P. gingivalis* and *A. actinomycetemcomitans* serotype a were elevated in the lesions

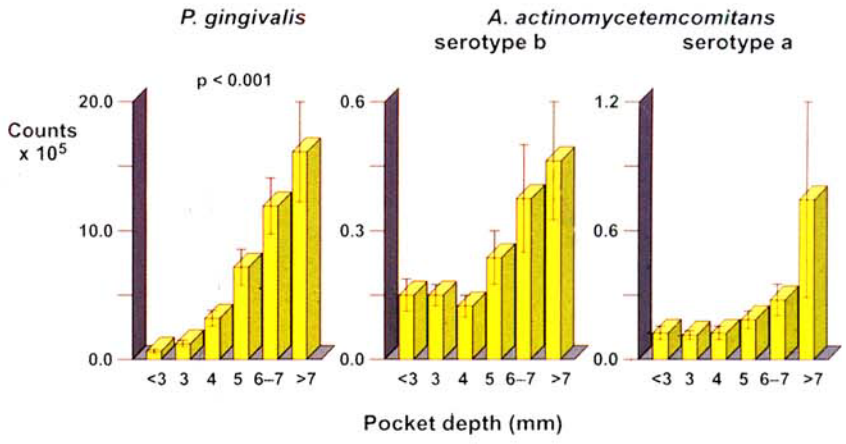

Fig. 4. Bar chart of the mean counts ($\times 10^5$) of *P. gingivalis* and *A. actinomycetemcomitans* serotypes a and b at sites that exhibited different pocket depths. The mean counts for each of the taxa at each pocket depth were averaged for a subject and then averaged across subjects. Significance of differences among pocket depth categories was evaluated using the Kruskal-Wallis test and adjusted for 40 multiple comparisons. The vertical bars and whiskers represent the mean±SEM.

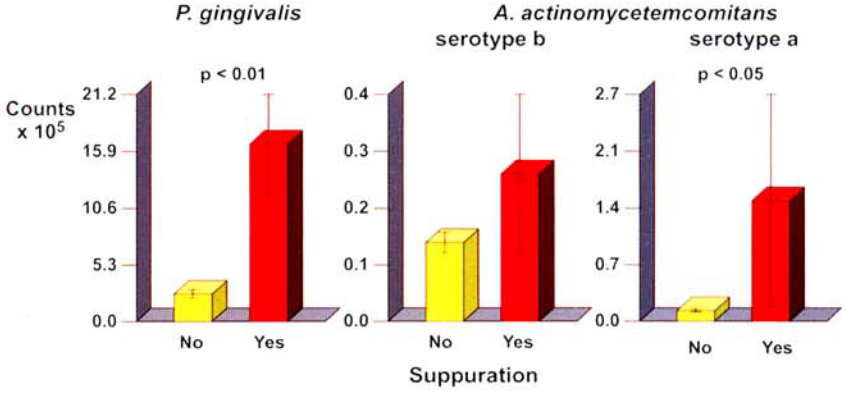

Fig. 5. Bar chart of the mean counts ($\times 10^5$) of *P. gingivalis* and *A. actinomycetemcomitans* serotypes a and b at sites that did or did not exhibit suppuration. The mean counts for each of the taxa were averaged at sites that did or did not suppurate in each subject and then averaged across subjects. Significance of differences between suppurating and non-suppurating sites was evaluated using the Mann-Whitney test and adjusted for 40 multiple comparisons. The vertical bars and whiskers represent the mean±SEM.

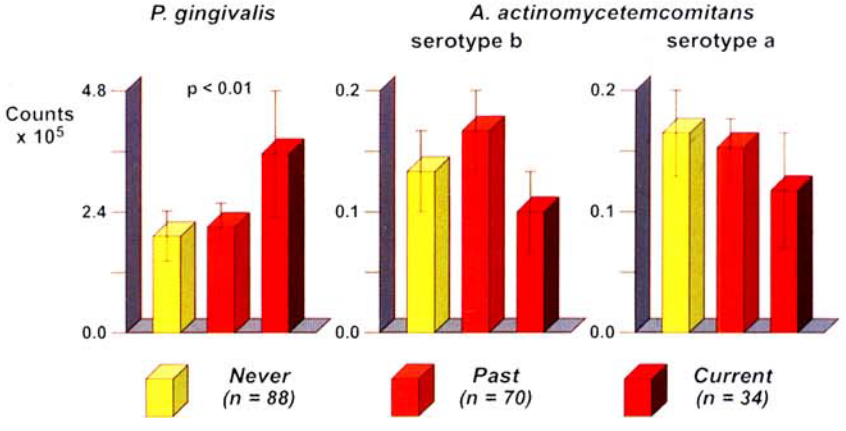

Fig. 6. Bar chart of the mean counts ($\times 10^5$) of *P. gingivalis* and *A. actinomycetemcomitans* serotypes a and b in subjects with different smoking histories. Subgingival plaque samples were taken from up to 28 sites in 34 current smokers, 70 past smokers and 88 subjects who had never smoked and evaluated for the presence and levels of 40 subgingival taxa using DNA probes. The mean counts for each of the taxa were averaged in each subject and then averaged across subjects. Significance of differences among smoking categories was evaluated using the Kruskal-Wallis test and adjusted for 40 multiple comparisons. The vertical bars and whiskers represent the mean±SEM.