Archs oral Biol. Vol. 27, pp. 75 to 77, 1982
Printed in Great Britain

0003-9969/82/010075-03$03.00/0
Pergamon Press Ltd

SHORT COMMUNICATION

BACTERIAL INTERFERENCE IN THE ORAL ECOLOGY OF *ACTINOBACILLUS ACTINOMYCETEMCOMITANS* AND ITS RELATIONSHIP TO HUMAN PERIODONTOSIS

J. D. Hillman and S. S. Socransky
Forsyth Dental Center, 140 The Fenway, Boston, MA 02115, U.S.A.

Summary—The plaque of 7 clinically healthy subjects was analysed for organisms inhibitory to the growth of *A. actinomycetemcomitans* strain Y4 on chocolate agar medium. Ten of the 11 sites harboured such organisms which constituted a median of 5.8 per cent of the total cultivable flora. Four to 30 per cent of the isolates in samples from eight sites in periodontitis were also inhibitory to strain Y4. By contrast, 15 of the 16 plaque samples from disease sites of 6 periodontosis (juvenile periodontitis) subjects showed no inhibitors. Four of 5 healthy sites sampled from 3 of the periodontosis subjects showed inhibitors in proportions similar to plaque from healthy subjects. The mother and 3 siblings of a subject with periodontosis lacked inhibitors in one or more plaque samples taken from their 1st molars. This work partially explains the basis for the localized pattern of destruction and the familial tendency of periodontosis.

Actinobacillus actinomycetemcomitans does not appear to be numerically dominant in the oral microbiota of individuals without adolescent destructive disease (Mandell and Socransky, 1981; Slots, Reynolds and Genco, 1980) but can attain proportions as high as 70 per cent of the cultivable microbiota in sites of advanced tissue destruction. Our purpose was to determine if microorganisms resident in healthy periodontal sites or ones affected by various forms of disease might prevent the establishment or growth of *A. actinomycetemcomitans*.

Subgingival plaque samples were obtained from the first molar regions of healthy people, ones with periodontosis (juvenile periodontitis) or adult periodontitis and from healthy gingival areas in the diseased subjects using the methods of Newman and Socransky (1977). The samples were dispersed by sonication for 10 s with a MSE sonic oscillator and serial 10-fold diluted samples spread on trypticase soy agar (Baltimore Biological Lab. Cockeysville, Md.) plates supplemented with 5 per cent sheep blood. The plates were incubated for 3–4 days at 35°C in an atmosphere of 80 per cent N_2, 10 per cent CO_2 and 10 per cent H_2. Colonies which arose on this medium were tested for their ability to inhibit the growth of *Actinobacillus* as follows: a lawn of *A. actinomycetemcomitans* strain Y4 was prepared on a chocolate agar medium by cross-streaking 0.2 ml of an overnight culture diluted 1:1000 with phosphate-buffered saline (pH 7). One hundred to 500 random isolated colonies obtained from the plaque samples on blood agar plates were replica-stabbed using sterile toothpicks onto chocolate agar plates with and without a lawn of strain Y4. The plates were then incubated for 2 days at 37°C in an atmosphere of 80 per cent N_2, 10 per cent CO_2 and 10 per cent H_2. Interference was regarded as present when direct visual examination revealed a zone of complete inhibition measuring 2 mm or more surrounding the stab. Results are expressed as the percentage of plaque isolates tested which inhibited the growth of strain Y4.

Plaque samples obtained from seven subjects ranging in age from 18 to 27 yr and who were free of clinically evident periodontal pathology, were analysed for their content of inhibitory organisms to *A. actinomycetemcomitans*. All but one of the 11 sites sampled harboured such organisms (Table 1). Plaque samples were obtained from two subjects, aged 19 and 31 yr, with generalized severe periodontal destruction with clinically obvious inflammation (adult periodontitis). Plaque recovered from deep pocket areas, as well as clinically normal sites, harboured organisms inhibitory to strain Y4 (Table 1).

By contrast, 15 of 16 plaque samples obtained from disease sites of six periodontosis subjects, showed no evidence of the presence of inhibitors. Five of these subjects showed clinical manifestations of uncomplicated periodontosis. The sixth showed a characteristic Papillon LeFevre syndrome. Four of the 5 clinically healthy sites in 3 of these subjects showed the presence of inhibitory strains in proportions similar to plaque samples from non-diseased subjects.

Plaque samples from the four first molars of the mother and 3 siblings of one subject with periodontosis were collected (Table 2). Plaque from the mother, who had advanced generalized destructive periodontal disease which had begun in adolescence, lacked inhibitory organisms in all of the 4 sites sampled. All the siblings, aged 7, 9 and 11 yr, lacked inhibitors in at least 1 of the 4 sites tested; in one sibling, all 4 sites lacked inhibitors.

The role of *A. actinomycetemcomitans* in adolescent destructive periodontal diseases remains to be established. Questions that arise are why the molar–incisor pattern of bone loss is so common and what is the underlying mechanism for the familial tendency of the

Table 1. Percentages of isolates inhibitory to *A. actinomycetemcomitans*

Sites	Healthy subjects	Periodontitis Diseased areas	Periodontitis Healthy areas	Periodontosis Diseased areas	Periodontosis Healthy areas
				0	
				0	
				0	
				0	
	0	30	10	0	0
	1.6	6	16	0	5.0
	2.8	6		0	8.4
	4.3	8		0	16.4
	5.3	4		0	44.0
	5.8	4		0	
	7.0			0	
	9.0			0	
	13.0			0	
	15.8			0	
	29.0			0	
				32	
Median	5.8	6	13	0	8.4
95 Per cent confidence interval of median	1.6–15.8	4–30		0–0	0–44*

* 93.75 Per cent confidence interval of the median; only 5 samples studied.
Differences between groups significant at $p < 0.001$ using Kruskal–Wallis test.

Table 2. Percentages of isolates inhibitory to *A. actinomycetemcomitans* in plaque from mother and siblings of a patient with periodontosis

Subject	Inhibitors (%)
Mother	0
	0
	0
	0
Sibling	23
	1
	0
	5
Sibling	0
	0
	0
	0
Sibling	42
	9
	7
	0

disease. These questions may be partially answered by our findings. If healthy individuals and individuals with other forms of periodontal disease harbour organisms in the gingival crevice area which are inhibitory to *A. actinomycetemcomitans* or other putative pathogens, it may be difficult for the pathogen to establish or achieve sufficient numbers to initiate or maintain local pathology. Whether *A. actinomycetemcomitans* is the pathogen in the affected adolescents or not, it seems clear that its presence in the lesion was not inhibited by associated microorganisms. Its absence or low numbers in individuals without adolescent destructive periodontal disease may be attributed at least in part to the presence of the antagonistic microorganisms. The mother and siblings each had one or more sites which lacked inhibitors. In the siblings, no overt periodontal pathology was evident. An understanding of the inhibitors, in terms of their nature and distribution, may explain the familial tendency and localized nature of periodontosis.

The mechanism of inhibition by the inhibitory organisms is not known. It does not appear to be acid formation because carbohydrate in the medium was limited and pure cultures of highly acidogenic organisms such as *Streptococcus mutans* were not inhibitory. Our findings provide some evidence for the widely held view that the presence of an inhibitory microbiota may serve to prevent infection by pathogenic oral microorganisms.

Acknowledgement—The investigation was supported in part by Grant DE-04529 from the National Institute of Dental Research.

REFERENCES

Mandell R. L. and Socransky S. S. 1981. A selective medium for *Actinobacillus actinomycetemcomitans*. *J. periodont.* In press.

Newman M. G. and Socransky S. S. 1977. Predominant cultivable microbiota in periodontosis. *J. periodont. Res.* **12,** 120–128.

Slots J., Reynolds H. S. and Genco R. J. 1980. *Actinobacillus actinomycetemcomitans* in human periodontal disease: a cross-sectional microbiological investigation. *Infect. Immun.* **29,** 1013–1020.