Salivary IgA in periodontal disease

FOLKE D. LINDSTRÖM & LARS E. A. FOLKE

Department of Medicine and Division of Periodontology, Center for the Health Sciences, University of Minnesota, Minneapolis, Minn., U.S.A.

Lindström, F. D. & Folke, L. E. A. Salivary IgA in periodontal disease. *Acta Odont. Scand.* 31, 31—34, 1973

The IgA content in whole and parotid saliva was determined in normal human subjects and in individuals afflicted with periodontal disease. The immunological measurements were made by means of a diffusion-in-gel method previously described. Elevated IgA-levels were found in the whole saliva of patients with periodontal disease, while normal levels of IgA were noted in sera and parotid saliva of all subjects studied regardless of their periodontal status. It was concluded that the elevated levels of IgA in the whole saliva was due to increased leakage of IgA via the gingival fluid.

Key-words: Saliva; periodontal diseases; immunochemistry; immunoglobulin; secretory IgA

Lars E. A. Folke, Division of Periodontology, School of Dentistry, University of Minnesota, Minneapolis, Minn. 55455, U.S.A.

Although periodontal diseases are poorly understood, it has been unanimously agreed that they are primarily infectious diseases caused by dental plaques. Presently, there is some information about the microbial composition of various dental plaques while information is lacking about host defense mechanisms relevant to periodontal disease processes.

The immunologic defense of mucous membrane surfaces has been studied by several investigators in recent years. (*Pierce*, 1959, *South et al.*, 1965). Several antibacterial or bacteriolytical factors, such as gammaglobulins and lysozyme (muramidase), have been thought to be involved. Among the three major immunoglobulins, IgG, IgA and IgM, it has been shown that IgA is the major antibody component of saliva. Yet, it comprises only about 15 % of the serum immunoglobulins (*Tomasi & Zigelbaum*, 1963; *Chodirker & Tomasi*, 1963). Most of the salivary IgA has a sedimentation coefficient of 11S, while serum IgA is 70S (*Tomasi et al.*, 1965). It was shown by *Hong* (1966) that this difference in size is associated with an additional antigenic fragment on the 11S variety. This additional fragment is often referred to as the »secretory» or »transport piece» because of the assumption that it is instrumental in secreting or transporting the IgA across the mucosal membrane.

Lehner and associates (1967) have reported low levels of salivary IgA in caries-prone persons. These results were first thought to indicate that IgA is associated with caries immunity. Even though such a correlation has not been

Received for publication, October 31, 1972.

observed in individuals with periodontal diseases, it is conceivable that an increase or a reduction of salivary gamma-globulin could affect the development and progression of such diseases. Because IgA is the dominant immunoglobulin contained in saliva, the study being reported here was undertaken to determine whether the levels of salivary IgA are different in patients with periodontal disease.

MATERIAL AND METHODS

The subjects used in this study were selected from among individuals seeking periodontal care at the Minnesota School of Dentistry. Following careful medical and dental examination, 19 subjects ranging from 17 to 53 years of age were selected on the basis of good general health, negative history of systemic infections and a periodontal disease index of 3 or above. The control group consisted of 12 persons, ages ranging from 22 to 37 years. The controls showed no evidences of periodontal disease and active caries. The severity of periodontal disease was assessed by the Ramfjord's (1959) Periodontal Disease Index (P.D.I.)

The flow of whole saliva was stimulated by chewing of paraffin wax and collected continuously at two separate occasions 1 week apart. The volume of saliva obtained during the first 30 minutes was measured and each sample was immediately centrifuged at 1500 rpm to eliminate heavy mucous material. Following a resting period of one hour, 20 ml of parotid saliva was collected directly from the Stenson's duct, using a Curby cap (*Curby*, 1953). Secretion was stimulated with sour candy (Regal Crown imported sour candy, Murray Allen Imports Inc., New York). The saliva samples were then dialyzed for

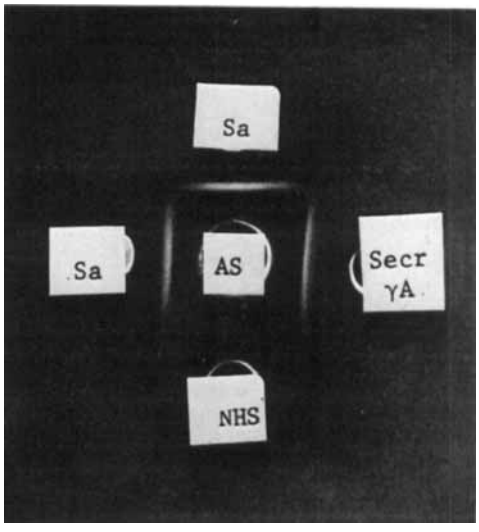

Fig. 1. Ouchterlony experiment showing transport-piece specificity of rabbit antiserum ag. colostral γA absorbed with concentrated normal human serum (AS). Sa = Saliva, Secr. γA = γA isolated from colostrum, NHS = Normal Human Serum

12 hours against running tap-water and lyophilized to concentrate the saliva 30 to 50 fold. A portion of the lyophilized material was then re-dissolved in saline and was used for Ig-quantitations.

Samples (10 ml) of the venous blood were obtained from all subjects at the time the saliva was collected.

Antisera towards 7S IgA were obtained by injecting rabbits subcutaneously at weekly intervals with an isolated IgA myeloma protein combined with Freund's complete adjuvant. 11S IgA was isolated from human colostrum according to the method of *Tomasi et al.* (1965) and were injected subcutaneously into rabbits together with Freund's complete adjuvant to produce antiserum. This 11S IgA was also used as IgA standard in Oudin-tube quantitation of salivary IgA. Absorption of antisera with serum from a patient with IgA deficiency made our antisera specific for IgA. The secretory IgA antiserum was also specific for the transport

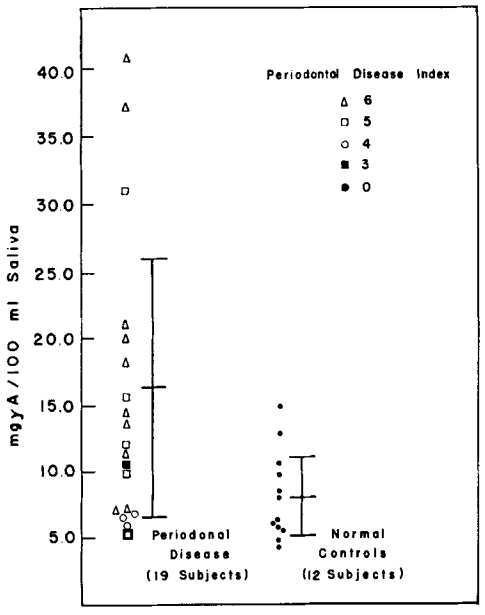

Fig. 2. Quatitation of IgA in whole saliva of subjects with and without periodontal disease. Median values and standard deviations are illustrated.

piece, as is indicated by the Ouchterlony experiment depicted in Figure 1.

The diffusion-in-gel methodology described by *Oudin* (1952) was utilized to quantitate the immunoglobulins. Colostral IgA was used as standard in quantitating salivary IgA, while 7S IgA was used as standard in estimating serum IgA.

RESULTS

Serum IgA levels were found to be within normal limits in all subjects studied, regardless of their periodontal status.

The IgA content in whole saliva was higher in the group afflicted with periodontal disease than in the control group, and the difference was statistically significant ($p < .003$) (Fig. 2). It is also evident from this graph that high salivary IgA levels correlated with advanced periodontal disease.

Table I. *IgA Concentration in parotid saliva collected from Stenson's duct (mg per 100 ml saliva)*

	Range of IgA quantity	Arithmetic mean
Periodontal disease group (n 19)	0—14.7	4.8
Control group (n 12)	1.1— 5.5	2.9

On the other hand, the IgA quantitation of parotid saliva did not reveal statistically significant differences between the two groups (Table I) when the data were analyzed using the rank sum test (*Dixon & Massey*, 1957).

The salivary concentrates were also examined in relation to the IgG content. Small amounts of IgG, ranging from 0.1 mg to 8 mg IgG per 100 ml saliva, were regularly found in samples of whole saliva, yet saliva collected directly from the orifice of Stenson's duct contained no measurable amounts of IgG. In general, samples of whole saliva from patients with periodontal disease showed higher IgG levels than did whole saliva collected from normal controls.

DISCUSSION

In this study, no evidence was found to indicate that periodontal pathology is associated with lower IgA levels in the saliva in comparison with normal subjects, as has been reported in individuals with active caries (*Lehner*, 1967). On the contrary, we observed higher level of IgA in whole saliva from patients with periodontal disease, as compared with normal persons. However, when the same kind of IgA quantitation was performed on parotid saliva, there was no difference between the two groups.

It seems likely that this is due to a considerable leakage of serum via the gingival pockets in patients with periodontal disease. The presence of IgG in whole saliva and its absence in parotid saliva, especially in the subjects with periodontitis, supports this interpretation. Since *Brill & Brönnestam* (1960) have shown that crevicular fluid contains at least seven different proteins that correspond to those in normal human serum, including gamma-globulin, it is likely that the increased leakage from the deepened gingival pockets contribute to the elevated levels of IgA in whole saliva.

Current theory concerning the etiology and pathogenesis of periodontal diseases involves an »interplay between exogenous insulting factors and varying host resistance» (*Hamp & Folke*, 1968). Although the role of salivary and serum IgA in this interplay remains difficult to assess with certainty, the results of this study do not exclude the contention that prolonged antigenic stimulus in an infectious condition like periodontal disease may stimulate the local IgA immune system.

Recently, *Holmberg & Killander* (1971) have demonstrated that the amount of gingival fluid increased with the severity of gingival inflammation and that the contents of different immunoglobulins conformed to that of serum. The absence of a »secretory piece» makes the authors conclude that the gingival fluid IgA was of serum type. *Berglund* (1971) has subsequently proposed that this IgA may also originate from cells in the inflamed gingiva.

On the basis of available information one might conclude, therefore, that locally produced IgA probably plays an active role in the periodontal disease process while *salivary IgA* produced by the parotid gland is likely of less importance.

Acknowledgements. This investigation was supported in part by Public Health Service Grant //DEO3174-02 from the National Institute of Dental Research.

REFERENCES

Berglund, S. E. 1971. Immunoglobulins in Human Gingiva with Specificity for Oral Bacteria. J. Periodont. 42, 9.

Brill, N. & Brönnestam, R. 1960. Immunoelectrophoretic Study of Tissue Fluid from Gingival Pockets. Acta Odont. Scand. 18, 95

Chodirker, W. B. & Tomasi, T. B., Jr. 1963. Gamma-globulins: Quantitative Relationships in Human Serum and Nonvascular Fluids, Science 142, 1080

Curby, W. A. 1953. Device for Collection of Human Parotid Saliva. J. Lab. & Clin. Med. 41, 493

Dixon, W. J. & Massey, F. J. 1957. Introduction to Statistical Analysis. McGraw-Hill, York, Pa.

Hamp, S. E. & Folke, L. E. A. 1968. The Lysosomes and Their Possible Role in Periodontal Disease. Odontol. Tidskr. 76, 353

Holmberg, K. & Killander, J. 1971. Quantitative Determination of Immunoglobuline (IgG, IgA and IgM) and Identification of IgA-type in the Gingival Fluid. J. Periodont. Res. 6, 1

Hong, R., Pollara, B. & Good, R. A. 1966. A Model for Colostral IgA. Proc. Natl. Acad. Sci. 56, 602

Lehwer, T., Cardwell, J. E. & Clarry, E. D. 1967. Immunoglobulins in Saliva and Serum in Dental Caries. The Lancet 1294

Oudin, J. 1952. Specific Precipitation in Gels and Its Application to Immunochemical Analysis. Methods Med. Res. 5, 335

Pierce, A. E. 1959. Specific Antibodies at Mucous Surfaces. Vet. Rev. Annotations 5, 17

Ramfjord, S. P. 1959. Indices for prevalence and incidence of periodontal disease. J. Periodont. 30, 51

Tomasi, T. B., Jr., Tan, E. M., Solomon, A. & Prendergast, R. A. 1965. Characteristics of an Immune System Common to Certain External Secretions. J. Exp. Med. 121, 101

Tomasi, T. B., Jr. & Zigelbaum, S. D. 1963. The Selective Occurrence of Gamma-IA Globulin in Certain Body Fluids. J. Clin. Invest. 42, 1552

South, M. A., Wollheim, F. A., Warwick, W. J., Cooper, M. D. & Good, R. A. 1965. Local Deficiency of Immunoglobulin A in the Saliva of Patients with Chronic Sino-Pulmonary Disease. J. Pediat. 67, 940