INFECTION AND IMMUNITY, Sept. 1985, p. 469–475
0019-9567/85/090469-07$02.00/0

Vol. 49, No. 3

Relationships between Levels of Lysozyme, Lactoferrin, Salivary Peroxidase, and Secretory Immunoglobulin A in Stimulated Parotid Saliva

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Received 27 December 1984/Accepted 30 May 1985

Recent studies suggest that salivary lysozyme (Lz), lactoferrin (Lf), peroxidase (Spx), and secretory immunoglobulin A (sIgA) may interact in a common antimicrobial system. A multiple protein approach therefore may be needed to determine the role of this system in oral health and ecology. In the present study we investigate the relationships between levels of Lz, Lf, Spx, and sIgA (adjusted for flow rate and total protein) in stimulated parotid saliva from 44 dental students. Principal components analysis was used to determine major patterns of intercorrelation between variables; cluster analysis was used to identify groups of subjects with similar salivary profiles for Lz, Lf, Spx, and sIgA. Spx tended to vary independently of Lz and Lf, which, in turn, tended to vary together. sIgA showed a weak negative relationship with Spx and a weak positive relationship with Lz and Lf. Six major clusters of subjects with similar antimicrobial protein profiles were found. These were significantly different at $P < 0.0001$. Spx was the most important determinant of cluster membership followed (in order of importance) by Lz, Lf, and sIgA. Cluster profiles were Spx^-, $sIgA\mu$, Lf^-, Lz^-; Spx^-, $sIgA^+$, $Lf\mu$, Lz^+; $Spx\mu$, $sIgA\mu$, $Lf\mu$, $Lz\mu$; Spx^+, $sIgA^-$, Lf^-, Lz^-; Spx^+, $sIgA\mu$, Lf^+, Lz^-; and Spx^+, $sIgA\mu$, Lf^+, Lz^+ (−, μ, and + refer to the position of the cluster mean for each variable relative to the overall mean for that variable). Results suggest that clusters may be a product of independent variation in the secretory activity of acinar and intercalated duct cells.

Saliva contains a variety of proteins which show antimicrobial activity in vitro. The best characterized are lysozyme (Lz), lactoferrin (Lf), salivary peroxidase (Spx), and secretory immunoglobulin A (sIgA) (18). The antimicrobial effects of these proteins have been defined primarily by experiments with oral bacteria (notably *Streptococcus mutans* and *Streptococcus sanguis*) in buffer systems. The nature of effects seen varies with experimental conditions. Thus, under appropriate circumstances, Lz functions enzymatically as a muramidase (13). However, as a cationic protein, Lz has also been shown to mediate bacterial aggregation (15) and adherence (L. M. Tellefson and G. R. Germaine, J. Dent. Res. **63**:188, 1983), to disrupt cell membranes (in association with inorganic ions) (27), and to activate bacterial autolytic enzymes (15). Lf can induce bacteriostasis by sequestering free iron (32), and it also displays an independent bactericidal effect (4). Spx, in the presence of H_2O_2 and SCN^-, can reversibly inhibit bacterial enzyme and transport systems by oxidation of protein sulfhydryl groups (with extended incubation this effect can be made irreversible) (35, 36). Finally, sIgA can specifically aggregate microorganisms and can prevent their adherence to oral tissues (it may also function as an opsonin for oral leukocytes) (1; S. Goldstine, A. Tsai, C. Kemp, M. Hanan, and M. Fanger, J. Dent. Res. **6**:247, 1983).

It is generally assumed that salivary Lz, Lf, Spx, and sIgA play a role in the maintenance of oral health and the regulation of oral ecology (18). However, clinical studies attempting to demonstrate such effects in vivo have yielded inconclusive results (18). A possible reason may be that most clinical studies investigate only one protein at a time. Even studies incorporating multiple proteins have generally considered each in isolation (3, 9). The problem with such an approach is that subjects with identical salivary levels of a given antimicrobial protein may be quite different with regard to other antimicrobial proteins, and those other proteins may affect the protein of interest in a variety of ways. Recent research shows substantial synergistic and antagonistic interaction among salivary antimicrobial proteins in vitro. Lf and nonspecific sIgA both have been shown to enhance the bacteriostatic activity of salivary peroxidase (34). On the other hand, specific sIgA has been shown to block the independent bactericidal effect of lactoferrin (8). However, specific sIgA may also enhance the bacteriostatic (iron-binding) effect of Lf (28, 32). Interactions between Lz and Spx, and Lz and sIgA have also been hypothesized, but have not yet been demonstrated (11, 27).

Little is known of interaction among Lz, Lf, Spx, and sIgA in vivo, but it is reasonable to presume that it occurs. The in vitro evidence suggests that interactive effects may vary with concentrations of the proteins involved; and salivary concentrations of Lz, Lf, Spx, and sIgA show considerable variation between persons (5, 7, 30, 33). It is therefore likely that patterns of interaction will also vary between individuals. Under such circumstances, attempts to relate quantitative variation in salivary concentrations of Lz, Lf, Spx, and sIgA to differences in oral health or ecology may require a multiple protein approach. Development of such an approach will require answers to two general questions. First, do salivary antimicrobial proteins vary independently of one another, or are they correlated? Complete independence would imply a unique pattern of interaction for every person, while complete correlation would suggest some mechanism of common control. Second, is it possible to define groups of persons who share similar salivary profiles of antimicrobial protein concentrations? Group

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members would presumably show similar patterns of interaction which would differ from group to group. The present paper addresses these questions within the context of a pilot study of Lz, Lf, Spx, and sIgA concentrations in stimulated parotid saliva samples from a dental student population. Multivariate statistical methods were used to describe relationships between salivary antimicrobial protein levels. Principal components analysis (26) was used to describe and interpret specific patterns of intercorrelation between Lz, Lf, Spx, and sIgA. Cluster analysis (21) was then used to identify subjects with similar values for these proteins. Results are discussed in terms of potential applications for hypothesis generation and testing.

MATERIALS AND METHODS

Saliva collection. The subject population consisted of 44 first-year dental students. Subjects were selected solely on the basis of their willingness to give informed consent and were prescreened only to the extent of deferring collection from persons observed to be chewing gum or eating. Samples were taken on Tuesdays between 10:00 a.m. and 12:30 p.m. from October through December 1983. Teflon Curby cups (19) with attached Biomedic tubing calibrated in four, 0.5-ml increments were used to obtain stimulated parotid saliva. Cups were placed over the papilla of the Stenson duct on the right side of the subjects' mouths. Pep-o-mint Lifesavers (Lifesavers Inc., New York, N.Y.) were administered as a salivary stimulus. Timing was initiated when 1 ml of saliva had accumulated in the tubing and continued until the first milliliter of saliva had drained into a marked test tube. At that point, timing ended, and the first milliliter of saliva was discarded. The collector was then removed, and the contents of the tubing were drained into a second test tube to yield a sample of approximately 2 ml, which was sealed and stored on ice. A maximum of eight saliva samples was obtained during any collection period.

Quantitation of salivary proteins. Actual volumes of saliva samples were measured to permit accurate calculation of flow rate. Assays for Lz, Lf, and sIgA were performed on the day of collection; Spx and total salivary protein were quantitated on the following day (samples were stored at 6°C at all times when not in use). Lysozyme was assayed by a modification of the lysoplate method (25). Plates were prepared from a mixture of 0.144% dried *Micrococcus lysodeikticus* cells (Sigma Chemical Co., St. Louis, Mo.) and 1% agarose in 0.066 M phosphate-buffered saline (pH 6.5). Standards were prepared from human milk Lz (Sigma) in concentrations from 0.25 to 2.0 μg/ml; 1% bovine serum albumin (Sigma) was added to all standards (25). A. 1.0-ml portion of each sample was acidified by the method of Papadopoulos et al. (24) to dissociate Lz bound to salivary mucins. Phosphate-buffered saline was then added to give a fivefold final dilution of acidified samples. Wells of 4-mm diameter were punched in the agarose-*M. lysodeikticus* gels, and 15 μl of standard or sample was added to each. Plates were incubated in a moist container for 18 h at 37°C. The zone of lysis diameters were then determined under conditions of dark-field illumination.

Lf was quantitated by rocket immunoelectrophoresis by the methods of Tabak et al. (33). The concentration of rabbit anti-human Lf (Calbiochem-Behring, San Diego, Calif.) in agarose gels was 1.67 μl/ml. Human colostral Lf (Sigma) in concentrations of 0.5 to 4.0 mg/100 ml was used as the standard. sIgA was determined by single radial immunodiffusion (17) with commercial LC Partigen IgA plates (Calbiochem-Behring). The standard was human colostral 11S IgA (Cappel Laboratories, Malvern, Pa.) in concentrations of 5 to 20 mg/100 ml. Salivary peroxidase was assayed spectrophotometrically with 2',2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) (Sigma) as a substrate (29). Change in optical density at 412 nm was determined for the first minute after the addition of H_2O_2 to the incubation mixture. Bovine lactoperoxidase (Sigma), at concentrations of 0.5 to 25.0 μg/ml, was used as the standard. Total salivary protein was quantitated by the method of Lowry et al. (16) with a bovine serum albumin standard in concentrations of 50 to 300 μg/ml (saliva samples were diluted 10-fold).

Data analysis. Sample concentrations of Lz, Lf, Spx, sIgA, and total protein were obtained by linear regression against standard curves. Distributions of scores were substantially skewed, as is often the case with salivary data (30). Skewness violates assumptions of statistical methods based on the normal distribution, and it may lead to misleading results when such methods are used (31). To remove skewness in the present data, concentrations were re-expressed as base 10 logs (31). Further adjustment was needed to remove variation in the data attributable to flow rate and total protein. Those variables tend to be highly correlated with concentrations of salivary proteins (19). In the present study, such correlation might obscure important relationships between the variables of interest. Data were adjusted for flow rate and total protein by a multiple regression approach (31). Log concentrations were first regressed against log flow rate and log total protein. Predicted values for log concentration were then subtracted from observed values to obtain residuals for Lz, Lf, Spx, and sIgA. The residuals represent a measure of the variation in salivary antimicrobial protein concentration that remains if the flow rate and total protein concentrations are held constant across all persons (31). Analysis of relationships between salivary antimicrobial protein levels was carried out in two stages. In the first stage, second-order partial correlation coefficients controlling for flow rate and total protein were calculated among Lz, Lf, Spx, and sIgA (these are equivalent to simple correlations between the residuals for those variables) (31). Principal components analysis was then used to identify major patterns of intercorrelation within the partial correlation matrix (26). For the second stage of the analysis, residuals for Lz, Lf, Spx, and sIgA were first standardized as z-scores. Groups of subjects sharing similar salivary antimicrobial protein profiles were then identified through repeated k-means cluster analysis by the method of Massart and Kaufman (21) for identification of robust clusters. The significance of the clustering solution found was tested by discriminant analysis, a procedure which also yields functions that can be used to classify unknown individuals (21, 26). All statistical analyses were conducted with programs from the SPSS (22) and BMDP (10) packages of statistical software.

RESULTS

Preliminary adjustment. Table 1 gives summary statistics for original and log values of flow rate, total protein, Lz, Lf, Spx, and sIgA. Measurements of skewness are included to show the effect of log transformation on data distributions. Simple correlations among salivary variables are presented in Table 2. As expected, antimicrobial proteins were significantly correlated with flow rate and total protein. Such variation was removed by the calculation of residuals (see above). Partial correlation coefficients obtained from the residuals appear in Table 3.

TABLE 1. Mean, standard deviation, and skewness values for flow rate, total protein, Lz, Lf, Spx, and sIgA in stimulated parotid saliva samples from 44 subjects

Variable	Mean ± SD	Skewness[a]
Original values		
Flow rate (ml/min)	0.20 ± 0.15	1.56
Total protein (mg/ml)	1.65 ± 0.57	0.24
Lz (μg/ml)	4.35 ± 4.30	3.36
Lf (mg/dl)	0.47 ± 0.47	2.34
Spx (μg/ml)	6.15 ± 2.49	0.01
sIgA (mg/dl)	14.91 ± 8.14	1.18
Log values[b]		
Log (Flow rate)	−0.81 ± 0.31	−0.12
Log (total protein)	0.19 ± 0.17	−0.64
Log (Lz)	0.51 ± 0.33	0.12
Log (Lf)	−0.50 ± 0.38	0.24
Log (Spx)	0.74 ± 0.22	−1.25
Log (sIgA)	1.12 ± 0.22	0.23

[a] Skewness is a measure of the extent to which distributions of scores deviate from normality (in a normal distribution skewness has a value of zero).
[b] Summary statistics computed from original values re-expressed as base 10 logarithms.

Principal components analysis. The partial correlation coefficients indicate that relationships among Lz, Lf, Spx, and sIgA persisted after flow rate and total protein were controlled for. However, the pattern of intercorrelation seen is difficult to interpret. A simplified description of the relationships was obtained by principal components analysis. The object of principal components analysis is to describe the variance represented by multiple variables in terms of a small number of major factors (26). The first principal component (PC) is the linear combination of the original variables that accounts for the greatest proportion of variance in a multivariate data set. The second principal component is statistically independent of the first, and it accounts for the next greatest proportion of total variance. There will always be as many principal components as there are variables, but the first few will generally account for most of the variance in a data set.

Table 4 provides the results of principal components analysis. The variable loadings indicate the correlation of original variables with each principal component (22). The eigenvalues are equal to the sum of the squared loadings for each principal component. They indicate the proportion of total variance accounted for by each PC. A PC must have an eigenvalue of 1.0 or greater to be considered significant (22). Communalities are the sum of the squared loadings for each variable across significant PCs. They indicate the proportion of variance in each variable that is accounted for by the significant PCs. In the present analysis, the first two PCs (PC1 and PC2) were significant. Together they accounted for about 72% of the total variance. They accounted for 77 to 89% of the variance in each of the variables, with the exception of Lz (39%). The pattern of loadings provided a simplified description of relationships among salivary antimicrobial proteins. The high loading of Spx on PC2 suggested that variation in Spx levels was largely independent of variation in Lz and Lf. Lz and Lf, on the other hand, appeared to be positively correlated, with both loading heavily on PC1. The relationship of sIgA to the other variables was complex, as it was positively correlated with PC1 but negatively correlated with PC2.

Cluster analysis. PC analysis can be used to describe an overall pattern of relationships among variables in a multivariate data set. Cluster analysis can group cases with similar values for those variables. For the present data, a stable clustering solution was obtained when the sample was divided into eight groups. The distribution of persons within clusters is presented graphically in Fig. 1 as a principal components display (21). This method provides a two-dimensional representation of variation in a multivariable space. A low score on PC1 suggests low Lz, Lf, and sIgA; a high score suggests high Lz, Lf, and sIgA. A low score on PC2 suggests low Spx and (to a lesser extent) high sIgA; a high score suggests high Spx and low sIgA. The plot shows that clusters 1 and 4 contained only two and one persons, respectively. Their small size made interpretation difficult, and they were not considered in further analyses. The six remaining clusters could be separated largely along the dimensions of variation measured by the principal components. Clusters 8 and 6 thus appeared to be low in Lz, Lf, and sIgA, with clusters 2, 3, and 7 being high in those proteins. PC2 further subdivided clusters into a high Spx group of clusters 8, 3, and 7 and a low Spx group including clusters 2 and 6. Persons in cluster 5 appeared to have mean values for all salivary antimicrobial proteins.

The PC display is useful in visualizing clusters and for describing general differences between them. However, it clearly did not account for all sources of differences among clusters, since clusters 3 and 7 showed considerable overlap. The overlap occurs because PC1 and PC2 accounted for only 72% of total variation in the sample. More precise descriptions based on 100% of total variation were obtained from cluster profiles (Fig. 2). A cluster profile presents the cluster mean for each variable relative to the grand mean for that variable. The profiles for clusters 2 and 6 confirm that both groups were low in Spx. Cluster 6, however, was at the mean for sIgA and below the mean for Lf and Lz. Cluster 2, on the other hand, was at the mean for Lf and high in sIgA and Lz. Cluster 5 was at the mean for all four variables, whereas clusters 8, 3, and 7 were all high in Spx. However, cluster 8

TABLE 2. Correlation matrix (Pearson r) for logs of salivary variables from 44 subjects

Variable	Log (Flow rate)	Log (total protein)	Log (Lz)	Log (Lf)	Log (Spx)	Log (sIgA)
Log (Flow rate)	1.0000	−0.2817[a]	−0.5884[a]	−0.4414[a]	−0.0948	−0.7704[a]
Log (Total protein)		1.0000	0.6217[a]	0.2100	0.6236[a]	0.3427[a]
Log (Lz)			1.0000	0.4736[a]	0.4017[a]	0.5951[a]
Log (Lf)				1.0000	0.3394[a]	0.5898[a]
Log (Spx)					1.0000	0.0440
Log (sIgA)						1.0000

[a] Statistically significant at $P \leq 0.05$.

TABLE 3. Matrix of second-order partial correlations (controlling for log [flow rate] and log [total protein]) between log (concentration) of salivary antimicrobial proteins in 44 subjects[a]

Variable	Log (Lz)	Log (Lf)	Log (Spx)	Log (sIgA)
Log (Lz)	1.0000	0.2936[b]	0.0990	0.1948[c]
Log (Lf)		1.0000	0.3488[b]	0.4272[b]
Log (Spx)			1.0000	−0.2280[c]
Log (sIgA)				1.0000

[a] Partial correlation coefficients are equivalent to simple correlations between residuals (31).
[b] Statistically significant ($P \leq 0.05$).
[c] Tending toward significance ($0.05 \leq P \leq 0.10$).

could be distinguished from 3 and 7 because it was below the mean for sIgA, Lf, and Lz. Clusters 3 and 7 were indeed similar. Both were at the mean for sIgA and high in Spx and Lf. The difference between them was a function of Lz, which was low in cluster 3 and high in cluster 7.

Each identified cluster had a distinct salivary antimicrobial protein profile. Statistical significance of the differences between clusters was evaluated by discriminant analysis (21). The overall differences between the six major clusters was highly significant ($P < 0.0001$). In pairwise comparison, each cluster was significantly different from every other at $P \leq 0.0007$ (these P values may have been exaggerated because the clustering process acts to minimize variation within clusters) (2). Spx was the single most important variable in discriminating between clusters, followed by Lz, Lf, and sIgA (in that order). The discriminant function program also yields classification functions (data not shown) which can be used to assign cluster membership to persons not originally in the sample. The effectiveness of classification functions can be estimated by using them to classify the original cases (22). A conservative estimate of classification function efficiency (10) for the present data was 88%, with most errors occurring between clusters 3 and 7.

DISCUSSION

One objective of the present study was to determine whether salivary levels of antimicrobial proteins are correlated. Findings obtained in this study suggest that variation in salivary levels of Spx is largely independent of variation in salivary levels of Lz and Lf, but that all three may co-vary to some extent with sIgA. The independence of Spx is interpretable in the light of recent histochemical studies of localization of Lz, Lf, Spx, and sIgA in salivary gland tissues. Lz and Lf appear to be primarily products of intercalated duct cells (14). Spx, on the other hand, appears to be primarily an acinar cell product (37). Transepithelial movement of sIgA is seen at many different sites, but intercalated ducts appear to be particularly active in this regard (14). Present results thus may be an indication that acinar secretion of Spx is regulated by a system which is independent from that controlling intercalated duct secretion of Lz and Lf. Combinations generated by the action of both systems in general could account for the pattern of clustering seen in Fig. 1. Additional hypotheses, however, are needed to account for low Lz levels in cluster 3 and for the complex relationship of sIgA with Spx, Lz, and Lf.

The role of sIgA is of particular interest in view of the findings of Arnold et al. (3) of elevated Lz, Lf, and Spx levels in saliva of patients with selective IgA deficiency. Similar elevations in the concentration of nonspecific antibacterial proteins have been reported for saliva of infants before the attainment of adult sIgA levels (I. D. Mandel, H. Turrett, and A. Alvarez, J. Dent. Res. **62**:217, 1983). Such findings suggest some mechanism whereby Lz^-, Lf^-, and Spx-producing cells are induced to compensate for temporary or permanent deficiency in the production or transport of sIgA. Evidence for a compensation response appears to be weak in the present data. An inverse relationship to sIgA levels was seen only for Spx, while the relationship of sIgA to Lz and Lf was positive. This pattern was largely attributable to clusters 2 and 8. Cluster 2 was low in Spx, but high in sIgA and Lz and at the mean for Lf. Cluster 8, on the other hand, was high in Spx, but low in sIgA, Lz, and Lf. The other clusters showed a comparable range of variation in Spx, Lz, and Lf, but sIgA levels in those groups did not differ appreciably from the population mean. This suggests that concentrations of Lz, Lf, and Spx were not strongly influenced by variation in sIgA levels. These findings are not necessarily inconsistent with the concept of a compensation response, since present results are based on a subject population of healthy young adults. It may be that compensation will only occur in cases in which the secretory immune system is grossly impaired or immature. Additional research will be needed to provide a definitive answer.

Additional research is also needed with regard to the second objective of this study, which was to define groups of subjects sharing similar profiles (and by implication, similar patterns of interaction) for salivary Lz, Lf, Spx, and sIgA. Cluster analysis did identify such groupings, but their reproducibility will depend on the extent to which salivary protein concentrations in the subject population are representative of salivary protein concentrations in the population at large. Dental students have advantages as a pilot population, but they may differ from the general population with respect to demographic variables such as age, sex, ethnic background, educational level, and socioeconomic status. Little is known of potential effects of such differences on salivary antimicrobial proteins, and consequences for current findings may be great or negligible. Another issue pertains to the size of the subject population. The present group was large relative to other studies in which multiple salivary proteins were incorporated (3, 9), but still small from the standpoint of statistical analysis (20). It thus is possible that the full range of potential variation in salivary levels of Lz, Lf, Spx, and sIgA was not encompassed here. Replication of the current study

TABLE 4. PC analysis of residuals for salivary antimicrobial proteins (adjusted for flow rate and total protein) for 44 subjects

Residuals	Loadings after varimax rotation[a]		Communalities[d]
	PC1[b]	PC2[c]	
Lz	0.62018	0.08135	0.39125
Lf	0.84309	0.24646	0.77153
Spx	0.21691	0.91897	0.89155
sIgA	0.71261	−0.56280	0.82455
Eigenvalues[e]	1.65772	1.22116	
Percent total variance[f]	41.4	30.5	

[a] Correlations between individual variables and PCs. Varimax rotation is a procedure for maximizing interpretability of loading patterns (22).
[b] First principal component.
[c] Second principal component.
[d] Sum of squared loadings across components.
[e] Sum of squared loadings across variables.
[f] Obtained by dividing eigenvalues by the total number of variables.

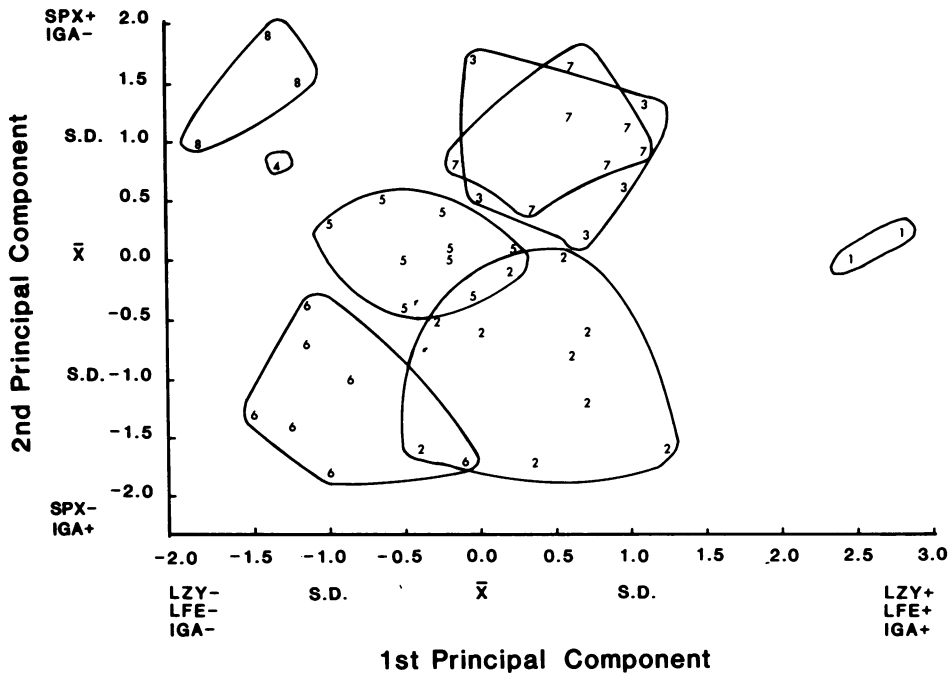

FIG. 1. Principal components plot of clusters obtained by k-means cluster analysis (21). Cases are plotted along axes defined by the first and second principal components. Principal components scores were obtained from SPSS subprogram FACTOR (22) and standardized as z-scores. Signs adjacent to variable names indicate the dimension of variation that is described by each axis. Eight clusters are plotted; the numbers assigned to cases denote cluster membership. Borders around clusters are provided to aid visualization of clusters; they have no statistical interpretation.

in a large, randomly selected sample representative of the general population is needed to clarify that point. Such a study will also provide an independent test of results obtained from principal components and cluster analysis.

Descriptive information from a population-based sample also can be applied to the question of continuity in cluster membership over time. If persons tend to move from cluster to cluster, then relationships between cluster membership and oral health and ecology are likely to be weak and difficult to define. Previous longitudinal studies of variation in salivary levels of individual antimicrobial proteins do suggest that variation within subjects across time is considerably less than the amount of variation seen between subjects at any time (6; J. D. Rudney, K. C. Kajander, and Q. T. Smith,

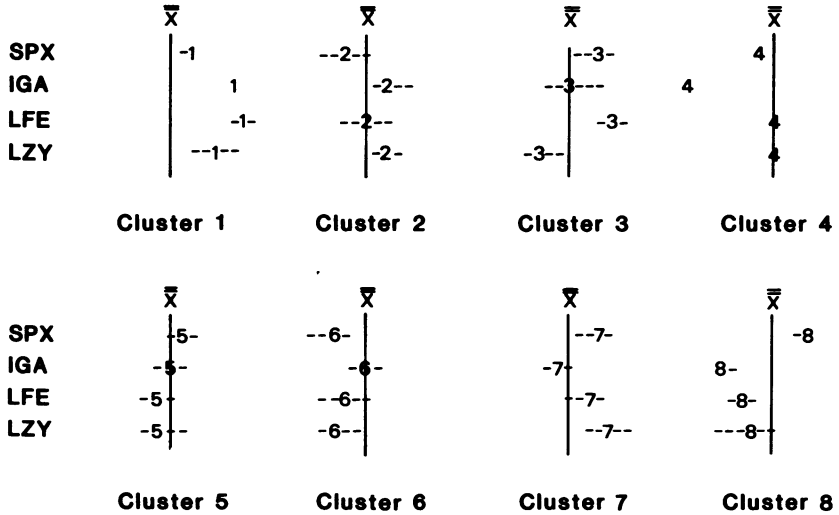

FIG. 2. Profiles of cluster means for standardized residuals (10). Cluster means are presented in relation to the grand mean for each variable, which is defined by the vertical lines (the z-transformation is used to standardize residuals, so that all variables have a grand mean of zero). Each column describes a cluster. Cluster numbers are printed at the location of the cluster mean for each variable. Dashes indicate 1 standard deviation above and below the cluster mean. Clusters 1 and 4 include only two and one individuals, respectively; the interpretability of their profiles is limited.

Arch. Oral. Biol., submitted for publication.). Additional support for such an interpretation is also provided by the data of Oberg et al. (23) on the longitudinal stability of densitometric patterns in polyacrylamide gels of parotid saliva proteins. Such patterns were found to be reproducible between different times of the day and on different days over periods extending as long as 12 months. The findings described above provide support for an hypothesis that persons will tend to maintain cluster membership over time. However, that hypothesis must be explicitly tested by prospective studies of subjects assigned to specific clusters.

If continuity can be shown, research then can proceed to empirical investigation of the biological significnce of cluster membership. Such an investigation could incorporate data from both clinical and experimental sources. Clinical investigation could focus on relationships between cluster membership and oral disease or on differences in the prevalence of specific oral microbes among clusters. Initial in vitro investigation could focus on comparison of saliva from members of different clusters with respect to viability (15), metabolic activity (12), aggregation (15), and adherence to hydroxyapatite (L. M. Tellefson and G. R. Germaine, J. Dent. Res. **63**:188, 1983) of bacterial species which do or do not colonize the oral cavity. Evaluation of in vitro differences among clusters should in turn lead to the generation of specific hypotheses regarding interactions among antimicrobial proteins. Such hypotheses could be tested by selective inhibition, removal, or enrichment of particular proteins (11). The information thus obtained could then be applied to development of a multiple protein model of the salivary host defense system. Evaluation of variation remaining within clusters likewise could lead to incorporation in the model of other important variables (these might include salivary levels of thiocyanate, relative degree of saturation of Lf, or titer of sIgA antibodies specific to particular antigens) (1, 4, 5, 8). Progressive refinement of such a model in turn should lead to improved understanding of the role of salivary antimicrobial proteins in oral health and ecology.

ACKNOWLEDGMENTS

We thank all the volunteers who agreed to participate in this study. We also thank Greg Germaine for comments on the manuscript, Roland R. Arnold for helpful discussions, and Lenette Baker and Judy Peterson for assistance in preparation of the manuscript and illustrations.

This research was supported by National Research Service Award 5 T32 DE 07014-09 from the National Institute of Dental Research, National Institutes of Health.

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