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Lymphocyte blastogenesis to plaque antigens in human periodontal disease

II. The relationship to clinical parameters

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This study undertook to correlate lymphocyte transformation to human dental plaque antigens with clinical estimates of periodontal disease.

Forty-eight patients with periodontal conditions ranging from normal gingivae to severe periodontitis were examined clinically. Oral cleanliness was determined by the Plaque Index System (Silness & Löe 1964) and gingival health was assessed using the criteria of the Gingival Index System (Löe & Silness 1963). Pocket depth and loss of periodontal attachment from the cementoenamel junction (Ramfjord 1959, Glavind & Löe 1967) were also measured. Triplicate microcultures of peripheral blood lymphocytes were stimulated with four different concentrations of human plaque antigens. The uptake of ^{3}H-thymidine during blastogenesis was measured by liquid scintillation counting.

There was no correlation between the stimulation by most of the isolated plaque antigens or pooled plaque and the periodontal conditions as determined by plaque index, gingival index, pocket depth and loss of attachment. However, there was a significant, although low, correlation between clinical parameters and the stimulation of peripheral blood lymphocytes by *B. melaninogenicus* and *A. viscosus*. Stimulation with *B. melaninogenicus* correlated more highly with pocket depth and loss of attachment, while stimulation with *A. viscosus* and *A. naeslundii* correlated more highly with plaque and gingivitis scores. The possible role of these organisms in the pathogenesis of periodontal disease involving cellular immunity was discussed as was the interpretation of data obtained in blastogenesis using peripheral blood lymphocytes and its relevance to the local phenomenon of delayed hypersensivity.

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Introduction

There is considerable evidence that cell-mediated immune mechanisms may be involved in the initiation and progression of gingival and periodontal lesions (Ivanyi & Lehner 1970, 1971a, Ivanyi, Wilton & Lehner 1972, Horton, Leikin & Oppenheim 1972, Nobreus, Attström & Egelberg 1974a, b, Lang & Smith 1976, 1977). This evidence has been derived largely from *in vitro* correlates of cell-mediated immunity (CMI). These assays are intended to reflect the systemic status of the host towards a particular immunogen, primarily as a measurement of immunologic memory. One of the most reproducible and quantitatively measurable assays is lymphocyte transformation (Thor 1968). However, the level of lymphocyte transformation may be affected specifically or nonspecifically by several fac-

tors. *In vitro* problems, including antigen dose-response relationships (Kiger, Wright & Creamer 1974, Lang & Smith 1976) and the addition of serum to culture (Ivanyi & Lehner 1971b), can alter results. Changes in the host's CMI due to aging (Gerber & Brown 1974, Weksler & Hütteroth 1974) can also invalidate findings from groups differing in age.

Most of the previous work dealing with CMI and periodontal disease has utilized laboratory grown stains of dental plaque microorganisms or extracts of pooled dental plaque. The significance of these antigens in terms of human periodontal disease can be questioned. Furthermore, if lymphocyte stimulation following antigenic challenge with a particular dental plaque antigen were to increase with increasing severity of periodontal disease as has been reported (Ivanyi & Lehner 1970, 1971a, Horton et al. 1972), the stimulation tests would be expected to correlate with all the clinical parameters used to assess periodontal condition providing these parameters truly reflect that existing periodontal condition.

The purpose of the present study is to correlate the blastogenic response of peripheral blood lymphocytes after stimulation with human dental plaque sonicates with the following clinical estimates of periodontal condition:

a) the amount of plaque present at the gingival margin;
b) the degree of gingival inflammation;
c) the depth of periodontal pockets;
d) the degree of loss of periodontal support.

Material and Methods

Forty-eight subjects in excellent general health, age 35–45 years, with at least 20 teeth were selected on the basis of availability during routine screening at The University of Michigan School of Dentistry. The patients had not received periodontal therapy, other than routine prophylaxis, for at least the last five years.

Following calibration of the examiner (N.P.L.) plaque was scored according to the Plaque Index System (Pl I) (Silness & Löe 1964) and gingival health was assessed according to the criteria of the Gingival Index System (GI) (Löe & Silness 1963). The depth of the gingival sulcus or the periodontal pocket (PD) was measured using a calibrated MI periodontal probe (Marquis Dental Mfg. Co., 2005 East 17th Avec., Denver, Colo 80206) and loss of periodontal support (LA) in relation to the cemento-enamel junction (Ramfjord 1959, Glavind & Löe 1967) was determined. All teeth present were scored on the mesiobuccal, distobuccal, facial and oral aspects and mean PII, GI, PD and LA were calculated for each participant.

At the time of the clinical examination, 25 ml of peripheral blood was collected from the antecubital fossa using a heparinized (Liquaemin Sodium, Organon Inc., West Orange, New Jersey) syringe.

Lymphocyte transformation test

The antigens were derived from *Veillonella alcalescens, Fusobacterium nucleatum, Bacteroides melaninogenicus, Actinomyces viscosus, Actinomyces naeslundii, Streptococcus sanguis* and three week old pooled dental plaque. Phytohemagglutinin (PHA-P) served as a positive control while buffered saline (pH 7.4) provided negative controls. The cultures were incubated, harvested and assayed for DNA synthesis according to the methods previously described (Lang & Smith, 1977).

Stimulation indices (SI) and Delta disintegrations per minute (Δ DPM) were calculated according to the following formula:

$$SI = \frac{\text{DPM antigen}}{\text{DPM saline}}$$

$$\Delta \text{ DPM} = \text{DPM antigen} - \text{DPM saline}$$

Table 1.

Regression analysis of the relationship between clinical parameters and SI

	F-statistics	Significance p value	Multiple R	S.E.
PHA	.7284	.5775	.2519	69.137
V. alcalescens	1.0746	.3809	.3015	2.458
B. melaninogenicus	5.7297	.0009**	.5896	.788
F. nucleatum	1.4639	.2298	.3462	1.890
A. viscosus	3.8907	.0088**	.5155	2.013
A. naeslundii	3.3405	.0181*	.4869	1.263
S. sanguis	.2899	.8830	.1620	1.136
Pooled plaque	1.1772	.3343	.3142	1.739

* $p < 0.05$ ** $p < 0.01$.

In order to correlate the clinical findings with the *in vitro* assay DPM, multiple regression analyses were performed for each patient's SI and Δ DPM for all antigens and PHA-P with all clinical parameters in all 48 subjects. Although dose response relationships can be derived from the data, only peak SI or peak Δ DPM are reported.

Results

The positive control assay showed a PHA-P stimulation which ranged from 1594 to 26441 DPM with a mean value of 6125.

Stimulation indices ranged from 25 to $>$ 300 with a mean value of SI = 105 indicating a positive assay for all the participants of the study. The background counts were generally below 80 DPM.

Since PII, GI, PD and LA are dependent on each other, they cannot be analyzed as independent variables. The results of regression analysis for the relationships between all clinical parameters and the SI is shown in Table I while Table II gives the corresponding values for Δ DPM. From these two Tables it can be seen that the only antigens which stimulated blast cell transformation in such a way as to correlate with any clinical parameters were *B. melaninogenicus, A. viscosus* and *A. naeslundii.* Only these three organisms, which showed

Table 2.

Regression analysis of the relationship between clinical parameters and Δ DPM

	F-statistics	Significance p value	Multiple R	S.E.
PHA	.4268	.7884	.1954	4088.7
V. alcalescens	1.4617	.2305	.3460	134.15
B. melaninogenicus	3.4192	.0163*	.4912	75.42
F. nucleatum	1.3924	.2527	.3389	143.04
A. Viscosus	4.6107	.0035**	.5479	126.88
A. naeslundii	2.7961	.0377*	.4543	87.62
S. sanguis	.2273	.9216	.1439	86.17
Pooled plaque	1.3452	.2688	.3335	87.85

* $p < 0.05$ ** $p < 0.01$.

Table 3.

Correlation coefficients and level of significance of the relationship between PII, GI, PD, LA and blast cell transformation for the Ag with significant regression analysis

	Correlation coefficients for SI and			
	PI I	GI	PD	LA
B. melaninogenicus	.4715**	.4902**	.5799**	.5618**
A. viscosus	.4977**	.4746**	.3188*	.3004*
A. naeslundii	.3441*	.3604*	.4165**	.2550 N.S.

	Correlation coefficients for Δ DPM and			
	PI I	GI	PD	LA
B. melaninogenicus	.3733**	.3760**	.4609**	.4838**
A. viscosus	.5245**	.4722**	.3359*	.3583*
A. naeslundii	.3817**	.3752**	.4288**	.3245*

* $p < 0.05$ ** $p < 0.01$ N.S. not significant

a significance in the regression analysis, were further analyzed.

The individual clinical parameters were then correlated separately with the SI or Δ DPM for all subjects. The results are shown in Table III. It is apparent that the correlation coefficients between all clinical parameters and blast-cell transformation are highly significant ($p < 0.01$) for *B. melaninogenicus*. However, the correlation coefficients between SI or Δ DPM and PD or LA seem to be higher (.5799 and .5618) than when these parameters are correlated to the PII and GI (.4715 and .4902).

For the two Actinomyces strains this relationship appears to be reversed. The correlation coefficients for PII and GI (.4977 and .4746) and the lymphocyte response are of a greater significance ($p < 0.01$) than those for PD and LA (.3188 and .3004) which are only significant at the 95 % level of confidence. Similar relationships exist for *A. naeslundii*. However, this organism does not show a significance between the SI and LA ($r = .2550$).

These findings are also illustrated in Figures 1 and 2. Figure 1a shows a scatter plot of the relationship between peak SI and PD for stimulation with *B. melaninogenicus*, while Figure 1b demonstrates the relationship between SI and LA for the same organism. Peak SI for *A. viscosus* are plotted against mean PII in Figure 2a and mean GI in Figure 2b.

It may be noted that although the multiple regression analysis of the relationship between stimulation of PBL with *F. nucleatum* and clinical parameters was not significant, there was a low but significant correlation ($p < 0.05$) between stimulation with *F. nucleatum* and PD (SI: $r = .3387$, Δ DPM: $r = .3757$).

All correlation coefficients of the relationship between any of the other clinical parameters and the rest of the antigens tested or PHA were not significant.

Discussion

If lymphocyte transformation responses following stimulation with dental plaque organisms were to reflect the periodontal disease status, a positive correlation would have to be found between clinical para-

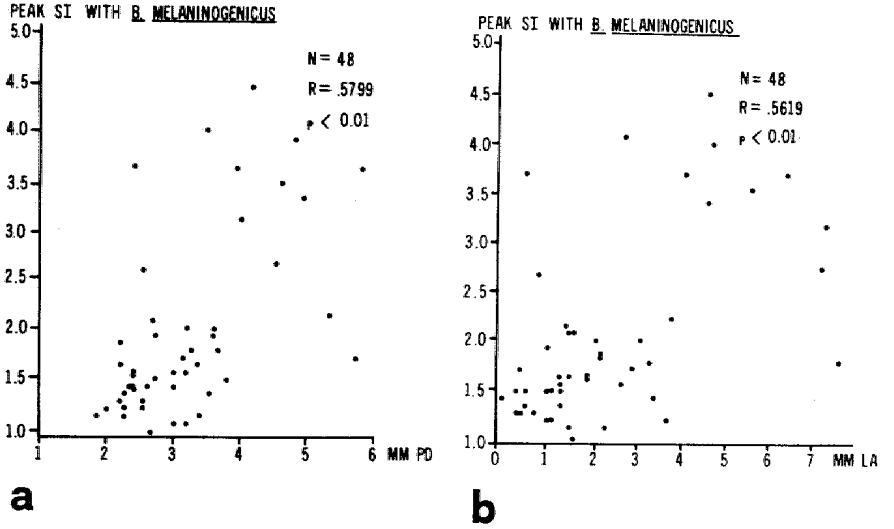

Fig. 1. Scatter plot of the lymphocyte transformation test with **B. melaninogenicus.** a) Relationship between peak stimulation indices (SI) and pocket depth (PD). b) Relationship between peak stimulation indices (SI) and loss of attachment (LA).

meters and the lymphocyte transformation test of a patient.

In this study there was generally no statistically significant correlation between the mean peak SI of most antigens in all subjects and their clinical parameters. These findings tend to support the conclusions of Kiger et al. (1974) who refuted the concept of linearly increasing SI with increasing deterioration of the periodontal tissues, proposed by Ivanyi & Lehner (1970, 1971) and supported by Horton et al. (1972).

Significant regression analysis were only found for SI with *B. melaninogenicus*, *A. viscosus* and *A. naeslundii*. However, the correlation coefficients with the clinical parameters were rather low (0.47–0.58 for *B. melaninogenicus*, 0.30–0.50 for *A. viscosus* and 0.26–0.42 for *A. naeslundii*). The fact that the SI with *B. melaninogenicus* showed higher correlations with PD and LA than with PII and GI merely supports the concept that this organism is predominantly associated with deep periodontal pockets (Lang & Smith 1977). This is not surprising when considering the nutrient requirements of this organism (Evans 1951, Lev 1958, Gibbons & MacDonald 1960). Likewise, the higher correlations of PII and GI with the SI obtained by stimulation with *A. viscosus* support an association of this organism with developing and established gingivitis (Lang & Smith 1977). *A. viscosus* and *A. israelii* were the only organisms which increased not only in bulk but in proportion during a recent experimental gingivitis in man (Loesche & Syed 1975, Syed, Loesche & Löe 1975).

This would support a concept of a possible pathogenic potential of this organism. If lymphocyte transformation tests were to serve as a satisfactory laboratory diagnostic

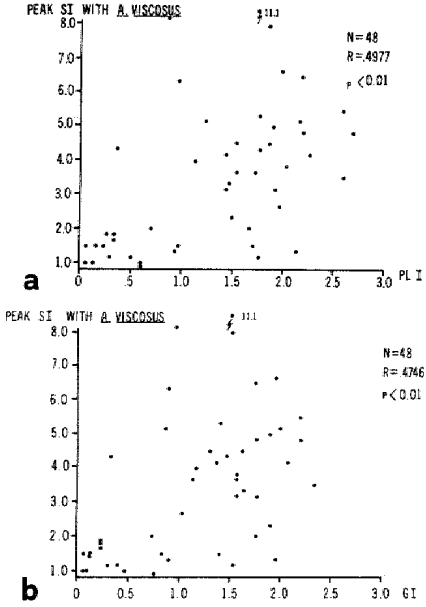

Fig. 2. Scatter plot of the lymphocyte transformation test with **A. viscosus.** a) Relationship between peak stimulation index (SI) and plaque index (PI I). b) Relationship between peak stimulation index (SI) and gingival index (GI).

test for the determination of the severity of periodontal disease in individual patients or populations, *A. viscosus* may be an important organism to use as an antigen.

Morphometric studies on chronic gingivitis lesions in man (Schroeder & Page 1972, Schroeder, Münzel-Pedrazzoli & Page 1973) have indicated that a marked increase of medium-sized lymphocytes is associated with a decrease in number of fibroblasts as the infiltrated connective tissue increases in size. However, it is unclear whether these cells are multiplying at the site of the lesion or are recruited from other sites. There is some evidence for the latter possibility (Mackaness 1971). Since the lymphocyte-fibroblast ratio of 0.8:1 for non-infiltrated connective tissue increases sixfold to 5:1 in infiltrated connective tissue of the gingival lesion (Schroeder, Münzel-Pedrazzoli & Page 1973), it is quite possible that cell-mediated immunological processes may influence the pathogenesis of periodontal lesions. Despite the fact that other mechanisms such as phagocytosis by macrophages (Parakkal 1969), enzymatic (Fullmer & Gibson 1966, Fullmer et al. 1969) as well as lysosomal activity (Freedman, Listgarten & Taichman 1968, Lange & Schroeder 1971) and bacterial products (Mergenhagen 1967) in association with the complement system (Snydermann 1972, 1973) may also contribute to the loss of periodontal supporting structures, cell mediated immune responses to some pathogenic organisms of the oral cavity have been demonstrated (Ivanyi & Lehner 1970, 1971a, b, Horton et al. 1972, Guggenheim & Schroeder 1974, Patters et al. 1976, Lang & Smith (1977). Therefore, lymphocyte transformation tests may have a potential of revealing the clinical condition of periodontal patients, if the relevant microorganisms are used as an antigen. Future research will have to concentrate on standardization of the assay systems to facilitate comparison of blastogenesis results from different laboratories (Patters et al. 1976).

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