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Lymphocyte blastogenesis to plaque antigens in human periodontal disease

I. Populations of varying severity of disease

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Lymphocyte transformation in response to dental plaque antigens has been used to determine the association between cellular immune responses and the periodontal status in man. An *in vitro* system for lymphocyte transformation was used. Triplicate microcultures of peripheral blood lymphocytes were stimulated with four different concentrations of *V. alcalescens, B. melaninogenicus, F. nucleatum, A. viscosus, A. naeslundii, S. sanguis* and pooled plaque of human origin. Phytohemagglutinin served as a positive and saline as a negative control. The uptake of ^{3}H-thymidine during blastogenesis was measured by liquid scintillation counting.

Forty-eight subjects divided into four equal groups of individuals with either normal gingivae, gingivitis, mild to moderate, or advanced periodontitis participated in the study. The subjects were all between 35 and 45 years of age. The peripheral blood lymphocytes of the normal subjects did not generally undergo blastogenesis with any of the organisms. However, when blastogenesis occured in other groups, the highest stimulation was found in the advanced periodontitis group. *B. melaninogenicus* stimulated blastogenesis only in the advanced group. *A. viscosus* and *A. naeslundii* stimulated lymphocytes to various degrees in all except the normal group. All the other antigens did not show any differences in stimulations between any of the groups.

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Introduction

Since the tissue response in periodontal disease is inflammatory in nature (Löe, Theilade & Jensen 1965) humoral as well as cellular immune mechanisms may be involved in its pathogenesis (Genco 1970, Genco et al. 1974, Horton, Oppenheim & Mergenhagen 1974, Taichman 1974). When dental plaque is allowed to accumulate on clean teeth surrounded by healthy gingivae a considerable bacterial load is eventually present to challenge the host defense mechanisms. Although cell-mediated mechanisms are responsible for host responses to a variety of microbial antigens (McCluskey & Leber 1974), the role of cellular immunity in the initiation and progression of periodontal disease remains unclear. Several assay systems including macrophage migration inhibition (David 1965), lymphocyte transformation (Dutton 1967) and cytotoxicity (Perlman & Holm 1969) have been proposed as *in vitro* correlates of cellular immunity. Numerous studies utilizing lymphocyte transformation tests have attempted to determine the role of cell-mediated immunity

in the initiation, development and progression of periodontal disease (Ivanyi & Lehner 1970, 1971a, 1972, Ivanyi, Wilton & Lehner 1972, Horton, Leikin & Oppenheim 1972, Kiger, Wright & Creamer 1974, Lehner et al. 1974). However, the results remain controversial. While some studies have proposed a direct linear relationship between blastogenesis of peripheral blood lymphocytes and the severity of periodontal disease (Ivanyi & Lehner 1970, 1971a, b, Ivanyi et al. 1972, Horton et al. 1972), others completely refute such an association (Kiger et al. 1974). Furthermore, it has been reported by one group that peripheral blood lymphocytes of patients with advanced periodontal disease do not undergo blastogenesis when challenged with bacterial antigens of dental plaque in the presence of autologous serum (Ivanyi & Lehner 1970, 1971a, b, 1972). This has been attributed to serum factors such as blocking antibody.

The purpose of this study is to evaluate the cellular immune response of patients of similar age with differing severity of periodontal disease by stimulation of their peripheral blood lymphocytes with various bacterial antigens isolated from human dental plaque of known age.

Materials and Methods

1. Experimental subjects and clinical procedures

Forty-eight subjects, aged 35–45 years and in good general health, each having at least 20 teeth, were selected on the basis of availability during routine patient screening at The University of Michigan School of Dentistry. No subjects had received any periodontal treatment, other than routine dental prophylaxis, within the last five years.

Plaque and soft deposits were suveyed according to the criteria of the Plaque Index System (Pl I) (Silness & Löe 1964). Gingival health was determinded by the criteria of the Gingival Index system (GI) Löe & Silness 1963). The depth of the gingival sulcus or periodontal pocket (PD) was measured with a calibrated Michigan No. 1 (MI) periodontal probe (Marquis Dental Mfg. Co., 2005 East 17th Ave., Denver, Colorado 80206). Loss of periodontal support (LA) was determined by measuring the distance from the cementoenamel junction (CEJ) to the apical base of the epithelial attachment (Sivertson & Burgett 1976, Ramfjord 1959, 1974, Glavind & Löe 1967). Measurements were rounded to the nearest millimeter. All teeth were scored on their mesial, distal, facial and oral surfaces.

All the clinical scoring was performed by one examiner. The intra-examiner errors for PD and LA for a single tooth surface measurement were $\pm$ 0.0042 and 0.0056 mm, respectively. The reproducibilities for Pl I and GI at a single site were 87 % and 92 %, respectively.

Mean Pl I, GI, PD and LA were obtained for each subject. Participants were then divided into four groups of 12 according to the criteria stated in Table I.

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

2. Preparation of the Peripheral Blood Leucocytes

Following sedimentation for 90 minutes at room temperature (20°C), the leucocyte rich plasma was removed, the cells centrifuged at 1000 rpm for 12 minutes and washed three times in Hanks Balanced Salt Solution (Difco Laboratory, Detroit, Michigan) containing antibiotics. The number of viable lymphocytes was determined by the dye exclusion test (Fallon et al. 1952) using 0.1 % trypan blue in a hemocytometer. The cells were resuspended in tissue culture

media TC 199 (Grand Island Biological Company, Grand Island, New York) with glutamine containing 100 units/ml Potassium Penicillin-G (Eli Lilly Manufacturing Co., Indianapolis, Indiana) and 0.2 mg/ml Streptomycin Sulfate (Eli Lilly Manufacturing Co., Indianapolis, Indiana). The pH was adjusted to 7.4 with TC sodium bicarbonate (Difco Laboratory, Detroit, Michigan). The cell concentration was adjusted 1×10^6 lymphocytes/ml TC 199.

3. Preparation of the Antigens

Plaque organisms were obtained during an experimental gingivitis study in humans as described by Löe, Theilade & Jensen (1965). These organisms were present in the predominant cultivable flora after a period of three weeks with no oral hygiene (Loesche & Syed 1975). The organisms were serially diluted, isolated and recultured. The purity of the cultures was checked microscopically and by growth on blood agar and mitis-salivarius agar plates (Difco Laboratory, Detroit, Michigan).

One litre of each pure bacterial culture was boiled in water for 60 minutes, centrifuged and washed three times in phosphate buffered saline (pH 7.2). The cells were then resuspended in 10 ml of ice cold phosphate buffered saline and ultrasonicated at 1.35 A for 30 minutes in a Branson B-12, 150 W sonifier surrounded by an ice jacket.

The ultrasonicates were centrifuged at 4°C for 15 minutes at 3000 rpm in a centrifuge with an angle head to remove residual whole cells. The supernatant was again centrifuged at 4°C for 90 min. at 14000 rpm. The resulting supernatant was used as the antigenic preparation.

Similary a preparation of pooled homologous plaque was obtained from as many sites as possible in four subjects after a three week period of no oral hygiene. Following dispersion in phosphate buffered saline (pH 7.2) the pooled plaque sonicate was prepared in a manner identical to that of the other antigens.

Four serial tenfold dilutions of the preparations were made in buffered saline and checked for sterility by anaerobic and aerobic culturing on blood agar for 72 hours at 37°C.

4. In Vitro Assay of Lymphocyte Transformation

Triplicate microcultures, each containing 2×10^5 peripheral blood lymphocytes (PBL) in 0.2 ml TC 199 and 10 % fetal calf serum (FCS) (Grand Island Biological Company, Grand Island, New York, lot No. 841619) were stimulated with four concentrations of each of the six antigens and pooled plaque. A total of 96 cultures were set up for each subject. Fetal calf serum used throughout the study was from the same lot. Bacto-PHA-P (Difco Laboratory, Detroit) at concentrations of 5.0, 2.5 and 0.5 μg/ml served as a positive control, while buffered saline (pH 7.4) was the negative control. The cultures were incubated for 78 hours at 37°C in a humid atmosphere with 5 % CO_2. For the final eight hours, 0.1 μc methyl ^3H-thymidine (sp. act. 6.7 Ci/mM, New England Nuclear, Boston, Massachusetts) was added to each culture in order to measure DNA synthesis.

Following harvesting using a multiple sample precipitator (Otto Hiller Company, Madison, Wisconsin), the cell cultures were washed twice with 0.9 % saline, precipitated on glass fiber filters (Reeve Angel, Clifton, New Jersey, No. 934AH) with 10 % trichlor acetic acid (TCA) and dried with 98 % methanol. After an overnight drying of the filters at 38°C, the cell cultures were processed for liquid scintillation counting, using a Beckman Counter LS-100C (Beckman Instruments, Inc., Fullerton, California). The counts per minute (CPM) were recorded and corrected for chemical quenching.

Table 1.

Clinical criteria for grouping PlI = Plaque Index, GI = Gingival Index, PD = Pocket depth in mm, LA = Loss of periodontal attachment in mm

Patient Group	Group Desig-nation	Pl I	GI	PD in mm	LA in mm
Normal	N	< 0.3	< 0.5	< 3	≤ 1.3
Gingivitis	G	≥ 0.3	≥ 0.5–1.6	≤ 3	> 1.3 ≤ 2.1
Moderate periodontitis	M	≥ 0.3	> 1.0–2.0	> 3 ≤ 3.8	> 1.45 ≤ 3.5
Advanced* periodontitis	A	≥ 2.0	> 1.4	> 3.4	> 2.6

* Subjects in this group were also required to have at least as many periodontal pockets ≥ 6 mm as there were teeth present.

Stimulation indices (SI) were calculated by dividing the mean disintegrations per minute (DPM) of the triplicate stimulated cultures by the mean DPM of the unstimulated saline controls. Delta DPM (Δ DPM) were also determined by deducting the mean DPM of the unstimulated cultures. Peak Δ DPM and peak SI of each antigen and PHA were then analyzed, using a univariate one way analysis of variance for all antigens and PHA in all four groups (i.e. normal, gingivitis, moderate and advanced periodontitis). Only SI > 2.5 were considered as evidence of a positive response.

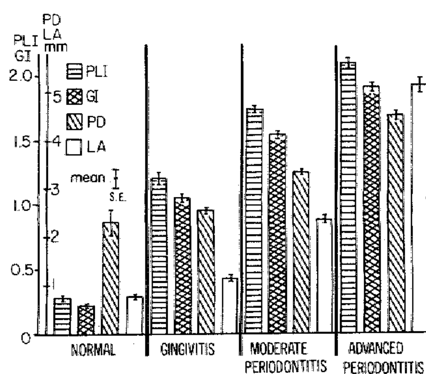


Fig. 1. Histogram of clinical parameters PlI = Plaque Index, GI = Gingival Index, PD = Pocket depth, LA = Loss of periodontal attachment.

Results

1. Clinical Examination

Since the placement of any of the 48 subjects into the four periodontal disease categories was determined by a strict set of prerequisites (Table I), analysis of the group showed highly significant differences in the clinical parameters used to determine the disease severity (Figure 1).

The mean age for the normal group (N) was 40.0 (S.D. 2.6) years, for the gingivitis group (G) 41.1 (S.D. 3.2) years, and for the moderate periodontitis patients (M) 40.3 (S.D. 3.2) years and for the advanced periodontitis group (A) 38.6 (S.D. 3.6). Since there was no difference between groups in age distribution, the influence of aging upon the lymphocyte response my be disregarded.

2. Analysis of Lymphocyte Transformation

The *in vitro* survival rate of human PBL was 70–80 % with this cell density of 1×10^6 lymphocytes/ml. The optimal synergistic effect of FCS on the blastogenic response of the PBL occurred at a 10–15 % concentration. A pulse labelling of 8 hours was found to be optimal for the uptake of ^3H-thymidine.

The background counts derived from unstimulated saline controls varied between 36 and 116 DPM. The mean background counts for Groups N, G, M and A were

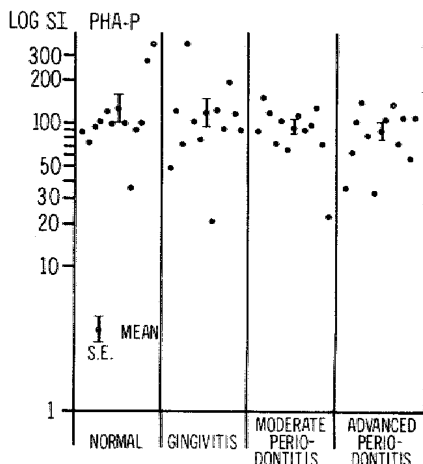


Fig. 2. Log Peak Stimulation indices (SI) of peripheral blood lymphocytes with PHA-P for all subjects.

58.8, 56.3, 65.4 and 69.3, resp. The differences were not statistically significant.

Stimulation of the cultures with PHA resulted in a mean peak Δ DPM for all 48 subjects of 6,125 (S.E. 575.6) resulting in a mean peak SI (PHA) of 105.9 (S.E. 9.86). There were no statistically significant differences in the mean peak SI (PHA) between any of the groups (Figure 2).

The results of stimulation of PBL with *Veillonella alcalescens* (V) antigen are shown in Figure 3a. Only one subject in group N displayed a SI > 2.5, while four from Group G did so. Seven subjects in group M, and six in group A also responded to *Veillonella* with SI > 2.5. However, a significant number of non-responders (SI $<$ 2.5) was seen in all groups. The analysis of variance revealed a trend to a significant difference between the mean peak SI (V) of Groups N and G with an F-statistics value of 4.0525. However, using the Scheffe Allowances at the 95 % level of confidence, these values were not significant.

Bacteroides melaninogenicus stimulated PBL to undergo transformation most frequently in group A. Nine subjects in that group showed SI > 2.5, while very little stimulation occurred in PBL from subjects in the other groups (Figure 3b). The mean peak SI in Groups N, G and M were 1.60, 1.54 and 1.69, respectively. However, Group A showed a mean peak SI = 3.15. This value was highly significant ($p < 0.001$) when compared to all the other groups.

Figure 3c reveals the response of the PBL cultures to stimulation with *Fusobacterium nucleatum*. No statistically significant differences were found between the response of any of the groups. However, in all four groups a small percentage of the patients responded to the antigen.

The response of all the subjects to stimulation with *Actinomyces viscosus* is shown in Figure 3d. While only one of the subjects in Group N displayed a SI > 2.5, 11 subjects did so in Group A. In each of the Groups G and M, eight subjects showed SI > 2.5. The mean peak SI for Group N of 1.66 increased to 4.19 in Group G, leveled off at 3.20 in Group M and increased again to 5.05 in Group A. The analysis of variance revealed a statistically significant difference between Groups N and G, and N and M ($p < 0.05$) and between Groups N and A ($p < 0.001$).

Actinomyces naeslundii did not stimulate PBL to undergo blast cell transformation in Group N. However, in Group G, seven, in Group M, five, and in Group A, seven subjects showed SI > 2.5. (Figure 3e). The mean peak SI for Group N was 1.30 and increased to SI = 2.45 and SI = 2.14 in Groups G and M.

Group A showed a mean peak SI of 3.04. The differences between the groups are only statistically significant between Groups N and A ($p < 0.05$).

The stimulation of PBL with *Streptococcus sanguis*, demonstrated in Figure 3f, shows no distinct pattern in any of the

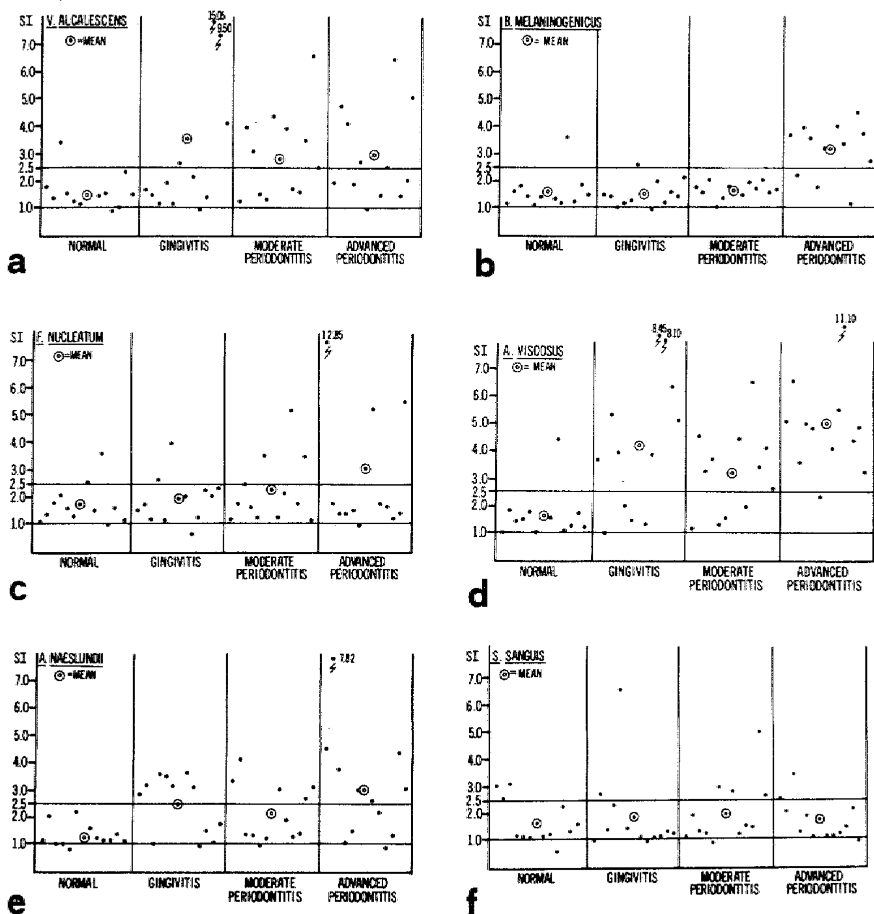


Fig. 3. Peak Stimulation indices (SI) of peripheral blood lymphocytes with human plaque antigens (all subjects): a) **V. alcalescens,** b) **B. melaninogenicus,** c) **F. nucleatum,** d) **A. viscosus,** e) **A. naeslundii,** f) **S. sanguis.**

groups. In Group N, three, Group G, two, Group M, four, and Group A, two subjects showed SI $>$ 2.5. The mean peak SI for Groups N, G, M and A were 1.65, 1.84, 1.96 and 1.71, respectively. No statistically significant differences were found between any of the groups.

Figure 4 shows the stimulation of PBL with *pooled plaque* antigen. SI $>$ 2.5 appeared to be randomly distributed in all four groups.

In Group N, two, Group G, three, Group M, two, and Group A, four subjects showed SI $>$ 2.5. The mean SI values were 1.86 for Group N, 2.06 for Group G, 1.67 for Group M and 2.90 for Group A and no

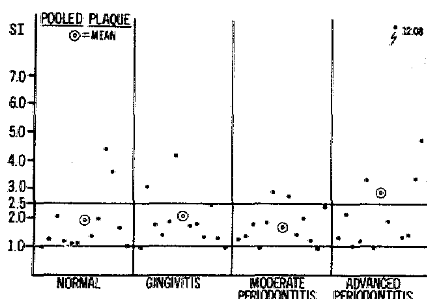
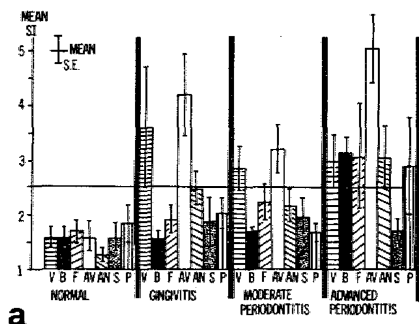


Fig. 4. Peak Stimulations indices (SI) of peripheral blood lymphocytes with three week old pooled plaque (all subjects).



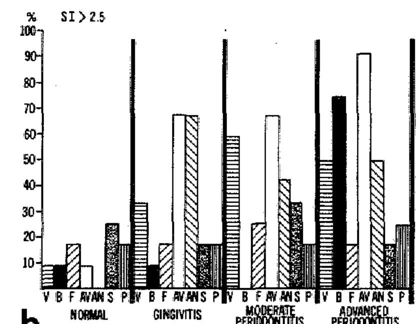


Fig. 5. **V = Veillonella alcalescens, B = Bacteroides melaninogenicus, F = Fusobacterium nucleatum, AV = Actinomyces viscosus, AN = Actinomyces naeslundii, S = Streptococcus sanguis, P = Pooled plaque.**

a) Mean peak stimulation indices (SI) for all antigens in all groups.

b) Percentage of subjects with a peak stimulation index greater than 2.5 (SI > 2.5) for all antigens in all groups.

statistically significant differences between any of the groups were found.

3. Overall Comparison of Stimulation with all Antigens

Figure 5a provides an overview of the mean peak SI and their S.E. for all the antigens tested in all four groups. In Group N, none of the antigens stimulated PBL to undergo blastogenesis. In Group G, most of the antigens did not trigger a significant response. Only *A. viscosus* (AV) demonstrated significant stimulation while *V. alcalescens* (V) and *A. naeslundii* (AN) showed a trend to significance. Similary, in Group M only *A. viscosus* showed a significant PBL response.

In general, the highest stimulation was seen in Group A. In this group all of the antigens, except *S. sanguis*, induced an average SI > 2.5. The highest overall mean peak SI was found in Group A with *A. viscosus* antigen. Group A was also the only group in which *B. melaninogenicus* stimulated PBL to undergo blastogenesis. *A. naeslundii* was the only other antigen causing a significant response.

Similar observations can be made if an analysis is made of the percentage of patients in each group responding at a SI > 2.5 to any particular antigen (Figure 5b). While the lymphocytes of only a very small percentage of subjects were stimulated with any of the antigens in Group N, the PBL of various percentages of subjects were stimulated in Groups G, M and A. With *B. melaninogenicus* a significant increase in the percentage of subjects with SI > 2.5 was noted in Group A (75 %). Another remarkable increase was noted with *A. viscosus.* With this organism only 9 % of the patients in Group N showed SI > 2.5. This percentage increased to 67 % in Groups G and M and to 92 % in Group A. *A. naeslundii* showed a less dramatic increase. The percentages of subjects responding to pooled plaque were identical in Groups N, G and

M (17 %), while about 25 % of the subjects responded in Group A.

Discussion

1. Methodology

Pilot experiments indicated that the viable count of PBL cultures decreased significantly between four and five days incubation. Therefore, three day cultures were used in this assay with the pulse labelling being on the fourth day. This time sequence of incubation corresponds with the one used by Ivanyi & Lehner (1970) but is significantly shorter than that used by Horton et al. (1972) and Kiger et al. (1974). The relatively poor survival rate of lymphocytes of 80 % after 4 days may be due to a high cell density (approx. 3×10^6 leucocytes/ml) in the culture media, resulting in pH changes and media depletion after 4–5 days.

The addition of serum or plasma to the PBL cultures definitely influences the system. Ivanyi & Lehner (1970) added 15 % autologous serum and Horton et al. (1972) 20 % autologous plasma to their cultures. Kiger and his co-workers (1974) used 10 % heat inactivated autologous serum. In order to minimize the effect of humoral factors and the complement system, heat inactivated fetal calf serum of the same lot was chosen in this study. The addition of 10 % FCS resulted in a five fold increase of the Δ DPM when PBL were stimulated with PHA.

A major difference between the culture system used by Ivanyi & Lehner (1970) and by Horton et al. (1972) and the present assay was the use of a microculture system. Persistently, triplicate cultures were stimulated with four different concentrations for each antigen or PHA and peak stimulations were calculated. Furthermore, there are major differences in the preparation of the antigens between this assay and others (Kiger, et al. 1974). Differences in culturing techniques and origin of antigens should not be disregarded when comparing results from different studies.

None of the plaque antigens appeared to stimulate PBL in Group N. It was, therefore, felt that a SI could be considered significant if it was higher than the mean SI + 2 S.E. for the antigen which stimulated to the highest level in Group N. The stimulation with pooled plaque resulted in a SI = 1.86 (S.E. 0.32). Since 1.86 + 2 (0.32) = 2.5, this value was used as the significant SI throughout the study. This level of significance is similar to that of other authors (Horton et al. 1972, Kiger et al. 1974). However, Ivanyi & Lehner (1970) refer to negativ stimulation with SI < 1.5.

2. Lymphocyte Transformation in Patients with Periodontal Disease

PHA stimulation is often used as the *in vitro* correlate of the cellular immunological responsiveness. In this study all the subjects showed significant stimulation with PHA. Within each of the four groups of normal, gingivitis, mild to moderate and advanced periodontitis, the variability in PHA stimulation is evident. Several *in vitro* factors, such as duration of culturing (Douglas, Kamin & Fudenberg 1969), as well as host parameters, such as aging (Gerber & Brown 1974) and disease state (Notkins, Mergenhagen & Howard 1970, Salaman 1970, Kauffman et al. 1974), may influence the responsiveness of T-lymphocytes to PHA. A marked increase of the peak response of PBL following stimulation with PHA during human experimental gingivitis has recently been associated with a shift of that peak response to lower PHA concentrations (Lang & Smith 1976). The present study does not reveal any significant differences between the different groups.

In general, the antigens tested did not stimulate PBL to undergo blastogenesis in the group of normal subjects. This is in

agreement with previous reports (Ivanyi & Lehner 1970, Horton et al. 1972) but disagrees with another report on lymphocyte transformation with plaque related and unrelated microorganisms (Kiger et al. 1974). Since the preparation of the bacterial extracts used as antigens in the Kiger et al. (1974) study and the culture conditions differed quite significantly from those used in this study, the results may not be comparable.

Ivanyi & Lehner (1970) and Ivanyi et al. (1972) presented almost linearly increasing values for the stimulation of PBL with *A. viscosus, V. alcalescens* and *B. melaninogenicus* with increasing severity of gingivitis and periodontitis. In this study it was impossible to reveal any linearly increasing values of stimulation of PBL with *A. viscosus, V. alcalescens* and *B. melaninogenicus* with increasingly severe disease. The lymphocytes of most of the subjects with gingivitis and mild to moderate periodontitis responded equally highly to *A. viscosus* and *V. alcalescens* and very little to *B. melaninogenicus.* The observation that increasing stimulation does not necessarily parallel increasing severety of the disease (Kiger et al. 1974) can be supported by the present findings, since nonresponders were found in all the groups and with all the antigens tested.

Increasing stimulation of PBL with pooled autologous as well as homologous plaque was reported by Horton et al. (1972) using two different concentrations of plaque. Likewise, Ivanyi & Lehner (1971, b) used autologous and homologous plaque for stimulation and obtained results similar to *V. alcalescens.* In the present assay system in which PBL were stimulated with pooled plaque, an association between the severity of the disease and the SI could not be established. In all groups of subjects there were a few responders but a significant number of non-responders. Since pooled plaque also contained all of the other antigens tested, similar results as, for example, *A. viscosus* could be expected. The fact that the SI with pooled plaque were not higher, may be attributed to a plaque preparation of poor antigenic potential.

The highest stimulation of the PBL cultures was generally seen in the group of subjects classified as advanced periodontitis. B. *melaninogenicus* triggered a high response when compared with the other groups of subjects. *A. viscosus* also stimulated PBL of almost all patients in that group. These findings do not parallel those of Ivanyi & Lehner (1970, 1971, a) and Ivanyi et al. (1972). However, they are in agreement with Patters et al. (1976) who also demonstrated stimulation of PBL with *A. viscosus* and *A. naeslundii* associated with gingival and periodontal inflammation. Stimulation of PBL with *B. melaninogenicus* was only seen in association with advanced loss of periodontal support.

Among the explanation offered by Ivanyi & Lehner (1970) for their results was that serum factors such as blocking antibody might have been responsible for depressing lymphocyte transformation in patients with advanced periodontal disease. A subsequent investigation (Ivanyi et al. 1973) did provide some evidence for this by finding restoration of response in some subjects when fetal calf serum was substituted for autologous serum. As the present study did not utilize autologous serum, the presence or absence of inhibitions could not be assessed. Other possibilities which might be considered include:

a) Antigenic dose response relationships appear to be of utmost importance in the proper evaluation of the responsiveness of lymphocytes (Anderson, Sjöberg & Möller 1972, Gronowicz, Continho & Möller 1974, Lang & Smith 1976). One predetermined antigen concentration indicates only one point on the dose response curve. This

response may miss the peak response, making proper evaluation difficult.

b) A significantly depressed response of PBL to mitogen (PHA) has been demonstrated with increasing age in the canine (Gerber & Brown 1974), as well as in humans (Weksler & Hütteroth 1974). Furthermore, Holm-Pedersen, Gaumer & Folke (1975) reported that elderly subjects would lack a response associated with gingival inflammation when their PBL were stimulated with lipopolysacharide from the cell walls of gram-negative organisms. The effect of aging on the immunocompetence of an individual should therefore not be disregarded. In the present survey an attempt was made to minimize the influence of age by selecting a population ranging between 35–45 years of age.

c) The source of the antigens and their preparation is obviously of importance. This study used human microbial isolates of known age.

3. Microbiological Considerations

It may be anticipated that *Bacteroides melaninogenicus,* owing to its unique nutritional requirements (Gibbons & MacDonald 1960, Gibbons & Engle 1964, Evans 1951, Lev 1958), would be found predominantly in association with deep periodontal pockets. *B. melaninogenicus* might be considered as a secondary pathogen rather than an organism initiating periodontal disease. The fact that *B. melaninogenicus* almost exclusively stimulated PBL of patients with advanced periodontal disease supports this concept. However, it has to be kept in mind that this organism may act as a pathogen by sensitization of the host's cellular immune mechanism, as well as by other mechanisms. This bacterium produces an intracellular collagenase (Gibbons & MacDonald 1961, Hausmann, Courant & Arnold 1967), which may also contribute to the connective tissue destruction occuring in progressive periodontal disease.

The second group of organisms which showed significant differences in their stimulation of PBL culture in the four groups were *A. viscosus* and *A. naeslundii.* The SI of the patients in Group G, M and A with *A. viscosus* and *A. naeslundii* were significantly higher than those of the normal group. Since *A. viscosus* was one of the two microorganisms which significantly increased in proportion to others in a recently reported bacteriological study of the experimental gingivitis model (Loesche & Syed 1975, Syed, Loesche & Löe 1975), our findings support the possibility of an association of this organism with developing and established gingivitis. *A. israelii,* the percentage of which also was significantly increased in the above mentioned study (Syed et al. 1975), was not tested in this study.

Within the limits of this study it may be concluded that certain isolated bacterial plaque antigens are capable of stimulating peripheral blood lymphocytes to undergo blast transformation. The lymphocytes of normal subjects generally show no or very little stimulation with dental plaque antigens. The highest stimulation of peripheral blood lymphocytes is generally seen in patients with advanced periodontal disease. The stimulation of lymphocytes with *B. melaninogenicus* occured predominantly in patients with advanced periodontal disease, which tends to associate this organism with deep periodontal pockets and loss of supporting structures, while *A. viscosus* was associated with gingival inflammation and advanced periodontal lesions.

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