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The American Academy of Pedodontics
Vol. 3, Special Issue

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Pathogenesis of gingivitis and periodontal disease in children and young adults

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Abstract

In adults and animal models, gingivitis consistently develops when bacterial plaque accumulates, and progresses sequentially through neutrophil, T-lymphocyte and B-lymphocyte/plasma cell dominated stages in a reproducible time frame. Periodontitis, also plasma cell dominated, develops at a later time on the same regime, but with time-variability and less than 100% consistency. Gingivitis rarely progresses to periodontitis in pre-pubertal children and seems to remain lymphocyte- rather than plasma cell-dominated. Bacteria are the accepted etiologic agents, with some particular species being associated with specific clinical features; however, definitive correlations have not been shown and a number of different species may be of etiologic significance in given cases. The signs of disease are more easily explained on the basis of activities of host response rather than solely to effects of bacterial enzymes or cytotoxins. Immunological responses have been implicated in this regard. Polyclonal, as well as antigen-specific, stimulation may be important. In studies of severe periodontal destruction in adolescents and young adults, dysfunctional PMN-chemotaxis has been associated with many cases, and B-cell hyperresponsiveness to polyclonal activation (which may be attributed to a T-cell regulatory defect), with some cases. Three working hypotheses are suggested: 1) periodontal disease presents as a well contained and regulated inflammation in children until around puberty, after which the usual, relative slow-progression of a B-cell mediated adult lesion is the rule; 2) exceptions to the self-containment in pre-puberty would be found in systemic disease states; and 3) exceptions beginning in young adulthood (rapid progression) would be found additionally in rather subtle functional aberrations of host defense.

Acknowledgments

Work at Virginia Commonwealth University, referred to in this review has been supported by grants DE-05139, DE 05054 and DE 04397 from the National Institute for Dental Research.

Introduction

The most common forms of human periodontal disease are gingivitis and periodontitis. Gingivitis is defined as an inflammation of the gingiva. The gingiva is all soft tissue surrounding the tooth coronal to the crest of alveolar bone and to a varying extent lateral to the bone, extending to the mucogingival junction. On the other hand, the definition of periodontium includes cementum, periodontal ligament, alveolar bone, and the gingiva; and periodontitis includes loss of attachment of periodontal tissues from the tooth and net loss of alveolar bone height.[1] Gingivitis is reversible, while regeneration after the destruction during periodontitis is not predictably achievable. Periodontitis in healthy children is not an extremely frequent occurrence. The most frequent periodontal disease in children, by far, is gingivitis.

Until quite recently, there was no information distinguishing gingivitis in children from gingivitis in adults, either clinically or histopathologically. It was perceived as a lesion confined to the marginal gingiva that might slow progress with age, although virtually no detailed study of the "juvenile marginal lesion" had been done.[2] Consequently, hypotheses of pathogenesis have arisen almost exclusively from study of adult humans and animals. Therefore, concepts of pathogenesis related to these studies will be reviewed briefly while considering emerging information related to children and young adults. Hypotheses of pathogenesis in children and young adults will be developed; however, definitive proof of disease mechanism(s) is lacking.

Clinical Studies of Disease Progression

The foundation for current concepts of pathogenesis of gingivitis lies in the now classic experimental gingivitis studies of Löe and coworkers.[3,4] The central observations that cessation of oral hygiene results in

gingivitis, and that resumption of oral hygiene reverts gingivitis to health, are critical indictments of the causative relationship of dental plaque to gingivitis. These observations have been confirmed repeatedly. The production of gingivitis in this model is universal among adult subjects, the only significant variable being the time necessary to reach a predetermined endpoint of gingivitis severity for each individual subject. This significant relationship of plaque bacteria to gingivitis has been buttressed further by demonstrations that prevention of plaque formation[5] or repetitive removal[6] also prevents gingivitis. Since the latter study involved children, the causative relationship of plaque to gingivitis is affirmed for children as well as adults.

Although the perceived irreversibility prevents ethical extension of this model to periodontitis in humans, analogous efforts in animals[7,8] indicate that continuance of the model for longer times results in periodontitis. This tends to reinforce the earlier presumptions based on epidemiological surveys which showed the amount of debris on the teeth to be the only significant correlate, other than age, of the severity of periodontal disease.[9-11] More recent longitudinal observations in human populations indicate much greater severity and rate of progression of periodontitis in human populations with poor oral hygiene, than in populations of the same age with good oral hygiene.[12] Clinical studies relating mechanical control of bacteria to successful periodontal therapy and prevention of recurrence[13-15] also support the concept of etiologic significance for oral bacteria in periodontitis as well as gingivitis. It is generally conceded that bacteria are the etiologic agents.[16-18]

There are very important differences, however, between the results of experimental gingivitis studies and efforts at natural induction of periodontitis. Whereas the former are uniformly effective in inducing gingivitis among adult individuals, only 80% of the dogs studied for four years developed periodontitis.[19] There are other confirmations that gingivitis does not invariably progress to periodontitis, and that it can persist for considerable time in some instances without such progression in adults.[20]

In children, progression to periodontitis is the exception rather than the rule.[2] There are also notable contrasts with respect to clinical development of gingivitis between children and adults documented in recent years. Mackler and Crawford[21] reported that six of eight children 3-5½ years of age failed to develop gingivitis during 26 days of an experimental gingivitis protocol. Another study of six children, 4-5 years old, compared with six male dental students, 23-29 years of age, confirmed a marked difference.[22] Further, the low tendency for development of gingivitis in the children in contrast to rapid development in the young adults was documented by the relatively objective measures of gingival exudate and bleeding units. In a cross-sectional investigation, young children exhibited a higher proportion of non-inflamed gingival units and less gingival fluid than did adolescents.[23]

Thus, there is definite suggestion at the clinical level for differences in pathogenesis between prepubertal children and older individuals. Differences in histopathology also exist, and will be discussed later in this review. If we accept dental plaque bacteria as the causative agents, variances in rates of progression and exceptions to progression could be explained either by differences in bacteria present or by differences in host responsiveness to the bacteria.[24]

Bacteriology

In contrast to earlier concepts, there is good evidence that all dental bacterial plaques are not the same. There are qualitative differences between plaques adjacent to healthy sites and those adjacent to diseased sites, and between supragingival and subgingival floras.[18] Healthy sites are associated with a predominantly gram-positive flora, with major representation of *Streptococcus* and *Actinomyces* species.[25-26] The flora adjacent to diseased sites has a higher representation of gram-negative rods and motile forms including spirochetes, with *Fusobacterium nucleatum* and *Bacteroides* species being among the most prominent representatives.[18,27-29] There are also suggestions of rather specific associations between certain microorganisms and specific periodontal conditions; e.g., a relative dominance of *Bacteroides assacharolyticus* (presumably now recognized as *B. gingivalis)* in highly inflamed destructive sites,[30] *B. melaninogenicus ss. intermedius* and *Eikenella corrodens* in destructive sites with minimal inflammation,[30] and *Capnocytophaga* species, *Actinobacillus actinomycetemcomitans* and other unidentified saccharolytic gram-negative rods in areas of severe destruction in young people.[30-31]

Recent reviews[32-33] have favored this concept of bacterial specificity in periodontal diseases. However, as concluded by others,[30] correlation of specific groups of organisms with certain clinical syndromes is not definitively established. Results of research in this emerging area of knowledge are highly method-dependent. Ongoing work in our clinics and laboratories in association with W. E. C. Moore and L. V. Holdeman attempts complete enumeration of the periodontal flora.[29,34-36] Results have been in general agreement with the previous findings of different flora in healthy and diseased states, and gram-negative organisms being more numerous than gram-positives in subgingival samples. However, more than 170 species have been differentiated from 73 samples. While 60% of the isolates belong to 54 previously described spe-

cies, the other 40% were members of 116 species which have not been described. Some of these, notably three species of the genus *Eubacterium,* occur frequently and in high numbers.[37]

Our data are consistent with the usual findings from mixed infections in various sites of the body, namely that each instance is an individual occurrence that may, or may not, be similar to others, and that there are probably common, less common, and quite unusual mixtures of bacteria that may be associated with any given periodontally diseased site.[29] Thus it is not yet possible to conclude that there are single, or a few bacteria, that are the specific etiologic agents for given periodontal diseases in adults. There are also problems in knowing whether particular bacteria associated with a diseased site contributed to the cause or are there because the disease created a favorable environment.

The bacteriology of periodontal disease in children has received very little study. There are reports that the incidence of *B. melaninogenicus* was found relatively rarely in children compared to adults or adolescents.[38-40] However, another paper reported *B. melaninogenicus* in all age groups studied, including ages 4-10.[41] Observations based on gram stain and morphology in smears of plaque during experimental gingivitis in children[21] were not very different from those previously reported for adults,[4] and *B. melaninogenicus* was recovered at least once from each child in the study. All of these reports were made prior to subspeciation of *B. melaninogenicus,* so their relevance to current reports from adult studies is difficult to assess. The question of whether differences in bacteria present account for the differences in clinical periodontal disease status between pre- and post-pubertal individuals remains open. It has barely been investigated.

Histopathology

The histopathological changes in gingivitis and periodontitis in adults and animal models have been studied intensively.[19,42-57] An extensive review of this information through early 1977 was published.[24] Page and Schroeder[20] divided the sequences of changes during the development of gingivitis and periodontitis into four stages, according to prominent histopathological signs. They termed these the Initial, Early, Established, and Advanced lesions. In health, the hallmark features in the gingival connective tissue are an even collagen density throughout the gingiva and an absence of clusters of inflammatory cells. In the initial lesion, present within two to four days after allowing plaque to accumulate, an increased volume of the junctional epithelium (JE) is occupied by polymorphonuclear leukocytes (PMN), blood vessels subjacent to the JE become dilated and exhibit increased permeability, a small cellular infiltrate of PMN and mononuclear cells has formed, and collagen content in the infiltrated area has markedly decreased.

In approximately four to seven days of plaque accumulation, gingivitis in humans evolves into the early lesion. The differentiating sign is the accumulation of large numbers of lymphocytes as an enlarged infiltrate in the gingival connective tissue. Associations between lymphocytes and cytopathically altered fibro-blasts are present. Earlier changes are quantitatively increased. By two to three weeks, the established lesion is present, characterized by the preponderance of plasma cells in an expanded inflammatory lesion with continuance of earlier features. The time frame of progression to the established lesion in adult humans seems quite predictable and reproducible. However, the established lesion may persist for variably long periods of time before becoming "aggressive" and progressing to the advanced lesion (periodontitis).

The infiltrate in the advanced lesion continues to be dominated by plasma cells. Collagen destruction has continued and loss of alveolar bone and apical relocation of the JE with "pocket" formation are now apparent. Throughout the sequence, viable bacteria apparently remain outside the gingiva, on the surface of the tooth and in the periodontal "pocket" against, but not invading, the soft tissue.

A notable finding by Longhurst and coworkers[58,59] is that the histopathology of chronic gingivitis in children does not correspond to the plasma cell-dominated, established lesion of the adult, but has an inflammatory infiltrate with a great majority of the cells being lymphocytes. This is most analogous to the early lesion as described by Page and Schroeder for the adult. Other reports on the nature of cellular infiltrates in various stages of periodontal disease had indicated that in mild gingivitis (early lesion?), the predominant lymphocyte was the T-cell, based on lack of cytoplasmic or membrane-associated immunoglobulin,[55,56] while in more severe gingivitis[56] and periodontitis[56,57,60] the B-cell line (of which the plasma cell is the mature end-cell) predominated. The implication may be that gingivitis in children is T-cell dominated, although this degree of delineation is not yet established.

Further argument may be made that conversion from a T-cell lesion to a B-cell lesion is the outstanding correlate of conversion from a stable to a progressive lesion.[61] Alternatively, since the major interpretation holds that there are plasma cell-dominated established lesions that do not progress for long periods of time,[20] there may be B-cell lesions that are progressive and those that are not. In either event, lesions that are progressive or have progressed are preponderantly B-cell; the only T-cell dominated lesion demonstrated thus far is within gingivitis, probably including the most prevalent lesion in children.

Pathogenetic Mechanisms

Because of the external location of bacteria, concepts of pathogenetic mechanisms have involved bacterial products or constituents rather than multiplication of bacteria within the tissue. In this context, although plaque bacteria can demonstrably produce potentially tissue-destructive enzymes and cytotoxins[17,62] which may be involved in pathogenesis, the nature of the infiltrate, rapidity of collagen destruction, and resorption of alveolar bone are more easily explainable by mechanisms of host response. The immune response has received much attention in this regard. The many effector systems evoked by an immune response provide attractive explanations for the inflammatory and tissue-destructive features of periodontal disease.[63] These features can be produced experimentally in animals on an immune basis.[64] Specific immune reactions, both T-cell and B-cell mediated, have been the usual explanation. Early reports of correlation between lymphocyte blastogenesis in response to oral bacterial preparations and the severity of peridontal disease,[65-67] followed by demonstrations of similarly stimulated release of bone resorbing factor(s),[68] collagenase, [69] and other mediators[69-71] provided support for classical T-cell mediated mechanisms.[72]

Many other studies have demonstrated lymphocyte blastogenic responsiveness to oral bacteria in gingivitis and/or periodontitis.[73-80] However, striking correlations with disease severity are not consistently found, except perhaps, in response to *Actinomyces* preparations in gingivitis and *Bacteroides* in periodontitis, and interpretations are clouded by the many inconsistent experimental variables among different studies. Although not all reports would agree, it would appear that periodontally "normal" individuals do respond to most stimulants used in these assays, and the conditions of the experiment may dictate whether quantitative differences are found between "normals" and other groups. Evidence from these studies does not relate strictly to T-cell mediated immunity, as both T- and B-lymphocytes have been shown to proliferate and produce lymphokines[74] including osteoclast activating factor.[81] The morphologic evidence discussed above suggests that destructive lesions are B-cell dominated.

Similar to classical T-cell mediation, circumstantial evidence is available for classical B-cell mechanisms (antibody and activated effectors), but convincing proof has been elusive. Many studies have demonstrated circulating antibody reactive with oral microorganisms,[82-94] but correlation with disease is not regularly found or convincingly remarkable.

A recent report, however, did indicate strikingly elevated antibody reactive with *A. actinomycetemcomitans* in juvenile periodontitis.[95] Extension and explanation of this finding may cause a re-evaluation of the role of antibody in periodontal disease. While immunoglobulins have been shown to be present in gingival plasma cells,[55,56,89,96-98] antibody specificity has been difficult to prove.[97] Antigens from dental plaque have been demonstrated in gingiva affected by periodontitis,[99] as have complement deposits,[89] but coincident localization of complement, antigen and antibody has not. Further, a recent morphologic and biochemical attempt failed to detect significant quantities of immune complexes in periodontal tissue.[100] Thus, a pathogenetic mechanism involving immune complexes, while theoretically attractive, has not been proven. Local anaphylaxis, mediated by IgE antibody, seems unlikely as a major mechanism because of the relative paucity of IgE in gingiva.[55,101]

Analysis of complement conversion products in gingival fluid did indicate that activation has occurred, possibly by antigen-antibody reactions as well as alternative pathway.[102,103] Overall, there is considerable circumstantial evidence that specific immunologic phenomena may mediate tissue damage in gingivitis and periodontitis, but there are also significant items of substantiation which are cloudy or missing.

The protective functions of the immune system should not be overlooked in these considerations; in fact, this feature plus other defensive capacities, such as provided by PMN activity, probably account for the fact that disease progression is generally quite slow in the face of a rather massive bacterial population.

It is apparent that antigen-specific immune responses are not the only means by which the efforts of the immune system can be induced. In contrast to the monoclonal activation in antigen-specific immunity, a role for polyclonal activation in the etiology of periodontal disease has been postulated. Clagett and Engel have reviewed polyclonal B-cell activation and speculated on its potential role in pathogenesis of inflammatory disease.[104]

Reports have indicated that lymphocytes from periodontally diseased subjects were more responsive than those from persons with a healthy periodontium when stimulated with levans, branched dextrans, and lipopolysaccharide.[105,106]

In a publication of work from our laboratories, strains of *B. melaninogenicus, A. naeslundii,* and *A. viscosus* were shown to have polyclonal B-cell activators (PBA) for human cells, and a hypothesis for the participation of PBA in periodontitis was developed.[107] Engel et al. had previously shown PBA activity for *A. viscosus* in murine systems.[108] We have studied to date, nine strains of gram-negative (five species) and gram positive (two species) bacteria commonly isolated

from periodontal microflora. Only one of these strains failed to function as a PBA. Potency, compared to a positive reference control (pokeweed mitogen), varied among strains tested, but some appeared as potent or more potent than the positive control. The magnitude of the response to a given PBA appeared to differ among individuals.

In addition to the features of the cellular infiltrate of gingivitis and periodontitis with which PBA-stimulated inflammation would be consistent,[104] bone resorption can be induced in *in vitro* systems by mitogenic (polyclonal) as well as antigenic stimulation.[81] In preliminary experiments, we have observed production of bone-resorbing factor(s) under the conditions of polyclonal activation by extracts of oral bacteria. Claggett and Engel[104] speculate that it may not be possible to implicate single etiologic agents where numerous species are present in close association with soft tissue, since many bacterial species possess PBAs. This would be consistent with the impressions gained from our bacteriological studies.[29] Combined effects of several PBA are also possible. Subsets of B-cells varying in their maturity are selectively affected by PBAs, and stimulation of immature B-cells can drive them to a maturational state in which they are susceptible to activation by a different PBA.[109-111]

Studies of Severe Periodontal Destruction in Adolescents and Young Adults

We have been studying individuals ranging in age from adolescence to 30 years, arbitrarily divided by clinical criteria into two populations. In one of these we have termed severe periodontitis (SP), further defined as the presence of 5 mm or more loss of attachment on eight or more teeth, not limited to first molars and/or incisors, in the presence of 6 mm or more of pocket depth and generalized gingival inflammation. The other group, termed juvenile periodontitis (JP), differs in that the severe periodontal destruction is limited to first molars and incisors, allowing for up to two additional teeth, and may involve fewer than eight total teeth. Both groups are free of systemic disease by history and signs. Studies are routinely performed in comparison to persons of the same age range with a healthy periodontium (HP).

1. *Lymphocyte function*

In investigations of the SP group, we have been unable to detect significant differences from HP in medical laboratory testing, immunoglobulin and complement levels, percent circulating B- and T-cells, serum antibody, lymphocyte blastogenesis, and lymphokine synthesis stimulated by a panel of bacteria, or phagocytic and microbicidal capacity of PMN. However, the SP group was significantly more responsive than the HP group to the polyclonal B-cell activator, staphylococcal protein A (SPA).[107] This hyperresponsiveness to PBA may be the reason these patients have had severe periodontal destruction at an early age, and gives additional reason to suspect that PBAs may be important factors in periodontal disease in general. SPA is a T-cell dependent B-cell polyclonal activator;[112] thus, the hyperresponsiveness could be due to aberrations in either T-cell or B-cell function. Also, the role of macrophages in polyclonal response needs to be clarified.

Preliminary data indicates a further characteristic of the SP group thus far in our results, that distinguishes them not only from the HP but also the JP population. Unstimulated peripheral blood leukocytes (PBL) from the SP subjects appear to incorporate significantly less ^{3}H-thymidine with time in culture than do PBL from either the HP or JP groups (Table 1). The increased uptake at days five and seven of culture by PBL from the HP and JP groups is consistent with a normal autologous mixed lymphocyte culture reaction (AMLR).[113,114] Thus, the significantly lower uptake by PBL from the SP group may indicate a suppressed or reduced AMLR. The normal AMLR is due to stimulation of T-cells by autologous non-T cells, [113-115] implying that failure to exhibit a normal AMLR can reflect abnormal T-cell responsiveness and regulatory function. This seems to be the case in systemic lupus erythematosus (SLE), wherein an impaired AMLR[116,117] and defects in induced suppressor T-cell function[118,119] have been found.

Our findings in the SP group are tenative as yet, in that we have not definitively identified our observations as AMLR, and the findings expressed in mean

Table 1. Thymidine uptake by unstimulated peripheral blood leukocytes after 3, 5 and 7 days of culture (CPM, Mean ± SE).

Subject* Group	N	3 Days	5 Days	7 Days
HP	16	4,319 ± 1,059	7,032 ± 1,475	17,577 ± 3,732
JP	8	3,215 ± 598	15,303 ± 3,467	26,647 ± 6,024
SP	13	3,222 ± 426	4,413 ± 540	9,285 ± 2,308

*HP - Healthy Periodontium, JP - Juvenile Periodontitis, SP - Severe Periodontitis.

data do not accurately describe every individual in the group. Nonetheless, these mean differences in the kinetics of ^{3}H-thymidine uptake in unstimulated PBL cultures, of the subjects studied so far, provide the clearest separation between clinical groups of any laboratory assay we have utilized. These findings, together with the hyperresponsiveness to SPA, provide a working hypothesis that there are individuals who suffer severe periodontal destruction at an early age because of a regulatory T-cell defect resulting in B-cell hyperresponsiveness.

A recent report indicates that the abnormalities in SLE are marked during active phases of disease and return to normal when disease activity decreases.[120]

That suggests that the loss of regulatory immune function in SLE expressed by suppressed AMLC is not a simple genetically determined trait, although an underlying genetic abnormality which requires a triggering, environmental event is not excluded. Should our investigations of the SP group confirm a defective AMLR, we will then want to know whether the apparent defect is reversible.

Regulation of B-cell response is quite complex. For example, the B-cell response to SPA in humans was found to be dependent on the activity of helper T-cell and regulated by the activity of suppressor T-cells,[112] and the magnitude of response may depend on the balance between helper and suppressor influences. Also, relative reponsiveness of helper and suppressor T-cells varies with concentration of the stimulant.

In addition to T-cell dependent polyclonal activators, there are T-cell independent, bacterially derived PBAs.[121] Soluble suppressors can be released on appropriate stimulation, which are suppressive for both T-cell dependent and T-cell independent mitogenic stimulation.[122] Further, T-cell helper activity for polyclonal B-cell responsiveness can be generated in response to antigen-specific activation of T-helpers.[123]

These regulatory systems provide reasons for variable responsiveness, under given conditions, in addition to the variable responsiveness of B-cell subsets and differences in relative potency of various bacterially derived PBAs previously mentioned.

The fact that gingivitis in pre-pubertal children normally does not seem to progress to B-cell dominance as it does in adults raises intriguing questions. Is this due to the type of stimulation received, or to regulatory influences? The answer(s) might aid in providing answers to why inflammatory lesions in adults progress to a destructive phase.

2. *Polymorphonuclear leukocyte (PMN) function.*

Although deleterious effects of PMN, through release of lysosomal hydrolytic enzymes, have received attention as potential contributors to pathogenesis of periodontal disease,[124,125] impairments of the defensive capabilities of the PMN would seem to be more important considerations in pathogenesis of periodontal disease in young people.

Quantitative PMN deficiencies, such as cyclic neutropenia[126,127] and chronic benign neutropenia[128-130] have been associated with severe periodontal destruction. Qualitative capabilities of the PMN relative to phagocytic function, including chemotaxis, pathogen recognition and ingestion, lysosome degranulation, and killing and digestion of microbes, may also have implications for the expression of periodontal disease if functional impairment exists. For example, Chediak-Higashi syndrome in both animals and man has been associated with abnormalities of PMN function, including depressed PMN chemotaxis, with severe periodontitis.[131-133]

Recently, PMN chemotactic responsiveness has been investigated in cases of juvenile periodontitis. The term "juvenile periodontitis" has been associated with the term "periodontosis.". Periodontosis was used to describe a non-inflammatory, degenerative lesion, generally occurring in relative absence of deposits on the teeth and leading to migration, loosening and exfoliation of the teeth.[134] This concept has been largely discarded for lack of supportive evidence.

As referred to earlier in this review, some have associated a rather specific microflora to juvenile periodontitis.[31-32] Our own studies to date are not conclusive with regard to whether there is a flora distinct from other peridontal diseases, but we do not routinely find the same organisms suggested by others to be prominent so far.[34] For example, our studies would indicate *Capnocytophaga* species to be almost exclusively supragingival in location.

Rather consistent findings have been reported by several laboratories, however, indicating depressed chemotactic responsiveness by PMN from individuals with juvenile peridontitis compared to PMN from periodontally healthy persons.[135-138] We also have conducted studies to observe the association between PMN chemotaxis (PMN-CTX) and severe periodontal disease in adolescents and young adults. Individuals from both the JP and SP populations defined above have served as subjects. We have routinely compared one or two such subjects with two HP subjects in an experiment conducted in a single day. Experimental conditions are similar to those previously described by Van Dyke et al.[138] In comparing 20 diseased subjects (12 JP, 8 SP), 16 experiments were performed. Data generated from a typical experiment are represented in Figure 1. The HP response to increasing concentrations of fMLP (5×10^{9} to 5×10^{7} M) is described by a bell-shaped curve with a maximum response at 5×10^{8} M. On this day the diseased subject's response curve was lower than, and dissimilar to, those of the HP

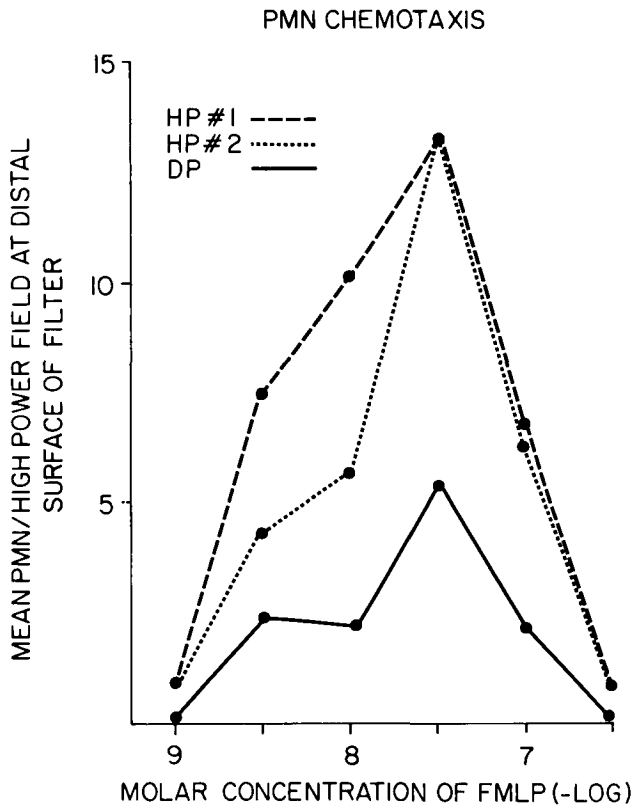

Figure 1. Neutrophil chemotactic response to various concentrations of formylmethionylleucylphenylalanine of three subjects - HP #1 = one periodontally healthy subject; HP #2 = second healthy subject; DP = young adult subject with sites of marked destructive periodontal disease. The responses represent the mean of three filters. The variance for each point was less than 10% of the mean.

subjects. Statistical analysis (analysis of variance) of all such experiments is presented in Table 2.

This analysis indicated that direct comparisons among daily studies were not possible because of the great variability among observations from experiments performed on different days (see DATE). The readily perceived fact of a relationship between concentration of fMLP and chemotactic response was confirmed (see DOSE), although the exact dose-response relationship did vary among experiments performed on different days (DOSE-DATE). However, the significant variable, SUBJECT-CATEGORY, (HP vs. JP & SP) and lack of significant variability due to Date-Subject Category interaction, confirmed that the diseased population did differ from the healthy population independent of the variability associated with experiments performed on different days. The moderately significant interaction of SUBJECT-CATEGORY-DOSE as a variable indicates that the subject comparisons are different at different concentrations of chemoattractant.

To permit between-diseased subject comparisons, given the above limits of variability, a cluster analysis

Table 2. Analysis of variance* chemotaxis results for 20 diseased subjects.

Source of Variance	P value
Date[a]	0.0001
Subject Class[b]	0.0001
Date-Subject Class	NS
Dose[c]	0.0001
Dose-Date	0.0001
Subject Class-Dose	0.0250

*Dependent variable = square root of the sum of the means of the filters. Each filter mean represents the number of PMN/field in 9 fields calculated to represent the entire filter.
[a]Day experiment performed [b]Healthy or diseased [c]Concentration of chemoattractant

of PMN-CTX data was used to analyze the heterogeneity within the population (Table 3). This analysis associated diseased subjects according to their PMN-CTX differences from HP subjects. The selection of three clusters most clearly separated the diseased population into distinct groups. Although cluster 1 appears to be elevated, or indistinct from HP's, clusters 2 and 3 are relatively chemotactically deficient, and cluster 3 was the least responsive group.

The majority of the tested diseased subjects fell into the "depressed" clusters, but the JP's were evenly distributed among the three clusters. With respect to dose relationships in this analysis, the dose at which the greatest difference from HP appeared was 10^{-7} M for 75% of the subjects in clusters 2 and 3 (10^{-7} M is generally one-half log greater concentration than that which results in peak chemotactic responsiveness).

Among the other 25%, maximum difference from HP appeared at various tested doses. Differences in apparent magnitude of the depression are present if data are analyzed according to the dose exhibiting maximum difference, compared to the mean of all doses, as shown in the table; a further difference in impressions of magnitude would occur if the peak chemotactic dose were chosen for analysis.

Thus, the variability associated with *in vitro* human PMN-CTX presents the most noteworthy challenge to interpretation of findings. The use of two "healthy" controls with each experiment in our study helped to reduce the impact of day-to-day variability and facilitated the observation that the diseased population was chemotactically deficient.

Alternative approaches include repetitive testing of subjects on different days, although Van Dyke et al.[138] reported that 9/54 comparisons among 18 pairs of

Table 3. Cluster analysis of PMN chemotaxis.[a]

Cluster No.	N. Subjects	Standardized Chemotaxis Difference[b]	Unstandardized % Response[c]	Clinical Group (N Subjects)	
				JP	SP
1	4	4 ± 2	167 ± 33		
		12 ± 3	123 ± 52	3	1
2	12	14 ± 1	66 ± 3		
		23 ± 2	43 ± 5	4	8
3	4	26 ± 2	47 ± 4		
		37 ± 4	28 ± 5	4	0

[a]Raw data from chemotaxis assays of PMN from diseased subjects was subtracted from that of healthy subjects performed on the same day for each concentration of chemoattractant.
[b]The upper number in each cluster is the mean difference for all concentrations of chemoattractant and all subjects in that cluster; the lower number is the mean of the greatest difference for each subject.
[c]The upper number in each cluster is the mean product (diseased/healthy) for all concentrations of chemoattractant and all subjects in that cluster; the lower number is the mean percent (diseased/healthy) using the data from concentrations resulting in the greatest difference for each diseased subject.

healthy individuals were significantly different (i.e., "false positives"), and only 8/32 diseased subjects always tested as deficient in repeat assays (26/32 were judged deficient). Because of the variability, expressions of data as percent deficiency should be viewed with caution with respect to *in vivo* biological relevance.

Nonetheless, similar observations from different laboratories, using different methods, lend strength to the conclusion that there is a deficiency in PMN chemotaxis among the population of young individuals with severe periodontal destruction. Based on the distribution of JP and SP among clusters according to chemotaxis in our work, and on the reports of others,[138] demonstration of a PMN chemotactic defect will not serve to clearly separate groups correlated with different clinical distributions of periodontal destruction. Neither does the fact of an association of chemotactic defect and periodontal destruction prove that the defect is a factor in pathogenesis. It is reasonable, though, to hypothesize that this does represent a weakened defensive capacity and might facilitate periodontal pathology.

Our findings with respect to lymphocyte function would suggest that the PMN defect is not the only aberration in defensive capacity or systems which may underlie some similar clinical syndromes in young adults. Other identified variables that may influence disease expression include serum factors[137,139] and antibody.[95] Defects in other cell types, such as monocytes, may also be found in young individuals with severe periodontal destruction.[140]

Periodontal Disease in Children with Systemic Disease

A review of periodontal disease pathogenesis in young persons must include acknowledgment of other systemic disease states having prominent manifestations in the periodontium (in addition to the neutropenias already mentioned). The particular relevance is that notable periodontitis in pre-pubertal children seems not to occur except in the presence of systemic disease. Among these are hypophosphatasia[141-143] and syndromes associated with dermatologic disorders such as Papillon-Lefevre syndrome.[144-146]

In hypophosphatasia a deficiency of alkaline phosphatase exists associated with a failure of cementum formation and resultant premature exfoliation of primary teeth. Papillon-Lefevre syndrome includes severe periodontal inflammation and bone destruction associated with hyperkeratosis of the palms of the hands and soles of the feet and occasionally other skin areas. Pathogenetic mechanisms are unknown. Reticuloendothelioses[146] and the leukemias[147] may have periodontal manifestations through infiltration of periodontal tissues by affected cells and concomitant altered defensive capacities.

Conclusion

The probable etiologic agents of gingivitis and periodontitis in children are bacteria, as in adults. Although specific bacteria have been implicated in some clinical syndromes in adolescents, this has not been shown beyond question. Very little information on the bacteriology related to periodontal disease of prepubertal children is available. In adults the pathologic features of established gingivitis and periodontitis are domianted by B-lymphocytes and plasma cells, while earlier features of gingivitis have a greater percentage

of T-lymphocytes. Most of the pathologic features of the destructive B-cell dominated lesion can be accounted for theoretically by immunological phenomena, both antigen-specific and polyclonal, and by consideration of functional attributes of other host response mechanisms. Many bacteria have polyclonal activation capacity, and polyclonal activation would be consistent with the possibility that many different bacterial species or combinations thereof may be of etiologic significance in given instances. In contrast to adults, gingivitis in pre-pubertal children seems limited to the lymphocyte-dominated stage (probably T-cell) without progression to plasma cell domination and periodontal destruction. An understanding of the regulatory mechanisms which result in this finding would be of major assistance in understanding pathogenesis of periodontal disease in general.

Those relatively rare instances of pre-pubertal severe periodontal destruction that do occur seem almost exclusively found when there is a concomitant systemic disease; e.g., Papillion-Lefevre syndrome, neutropenias.

Instances of severe periodontal destruction in adolescents and young adults have been associated with qualitative functional abnormalities, including defects in PMN chemotaxis, monocyte chemotaxis, and B-lymphocyte hyperresponsiveness, perhaps attributable to T-cell regulatory abnormalities.

As overall hypotheses, it would appear that: 1) periodontal disease in children and young adults normally presents as a well contained and regulated inflammation without a significant destruction until around puberty, after which the more usual, relatively slow, progression of the adult lesion is the rule; 2) functional abnormalities of defensive capacities provide for the exceptions of rapidly progressive destruction in adolescents and young adults; and 3) it is reasonable to postulate that the severity of the disease expression will relate to the severity of, or additive effects of, abnormalities of host defense.

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