Humoral immune responses in gingival crevice fluid: local and systemic implications

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General aspects of immunoglobulins in gingival crevice fluid

The oral cavity represents the entry portal for a wide array of antigenic challenges, both transient and more permanent. The more permanent challenges are represented by the substantial bacterial colonization that exists in the oral cavity. This environment presents a variety of niches for the establishment of unique microbial ecologies including the oral mucosa, tongue, tooth surface, and gingival sulcus. Many members of the complex oral microbiota maintain a symbiotic relationship with the host; however, select individual species are clearly pathogens (i.e. Streptococcus mutans and Actinobacillus actinomycetemcomitans) within this environment.

For the host to maintain homeostasis within the oral cavity, various immune response systems contribute to controlling the microbial colonization. These systems represent three interrelated entities and include the salivary, systemic (serum) and gingival tissue immune systems (Table 1). This review will focus primarily on the gingival tissue immune system, and gingival crevice fluid (GCF) as the biological material contributing to the local sulcal environment.

The oral epithelium represents the only site in the body in which the epithelial barrier is deliberately breached by hard tissues (i.e. cementum, enamel), which must be sealed from the external milieu. Even in gingival health, there is a fluid transudate that flows from the site of this seal, presumably as a mechanical factor is minimizing bacterial accumulation. This fluid also contains a variety of macromolecular components that are derived from serum and the interstitia of the gingiva. The concentration of these components appears to be related to the size of the molecules, suggesting a passive filtering system of the intact gingival tissues. The levels of these molecules, including protease inhibitors, β2-microglobulin, fibrinogen, albumin, and lipoproteins, appear proportional to serum levels. Similarly, immunoglobulin G (IgG) and IgA in a ratio comparable to serum are contained in this transudate. Thus, in addition to the mechanical cleansing action of the fluid flow, these innate and acquired immune molecules may contribute to homeostasis.

As inflammation of the gingiva increases, the transudate changes to an inflammatory exudate containing higher levels of serum-derived molecules, vascular derived cellular components of inflammation, and locally derived molecules from the gingival tissues. Since the macromolecules from serum and gingival tissues are identical species, it has been difficult to determine accurately the contribution of each to the exudate. The GCF also contains a variety of inflammatory and immune cells associated with increasing inflammation. The neutrophil comprises the majority of sulcal leukocytes irrespective of the stage of periodontal destruction. However, mononuclear white blood cells, including T cells, B cells and monocyte/macrophages, are also detected and appear to increase with increasing emigration of these cells into the inflamed tissues. In general, GCF contains approximately 95% polymorphonuclear leukocytes, 2–3% monocytes, and 1–2% lymphocytes (Table 2). Finally, both bacterial products that can modify host responses and host regulatory molecules that can alter the cellular distribution in the GCF and contiguous tissues can be detected.

There are clearly unique molecules that are produced in the local tissues. The GCF exhibits an ex-
ceedingly more complex array of immune components than does glandular saliva and the fluid flow contributes many antibacterial mechanisms to the oral cavity (24). The GCF is derived from gingival capillary beds (serum components) and resident and emigrating inflammatory cells. Both IgG and IgA are present in the GCF and are derived from serum and plasma cells in the gingival tissues. These immunoglobulins have been shown to have antigenic specificity for local bacterial antigens, as well as a substantial portion that appears to be derived from polyclonal B-cell activation. In addition, numerous studies have confirmed that significant local elevations in immunoglobulins occur in periodontitis resulting from extensive local production.

Immunoglobulins and antibodies of all isotypes are generally at low levels in GCF from healthy sites, minimizing the potential for various hypersensitivity reactions that could contribute to local tissue destruction. The level of inflammatory/immune mediators in sites of gingivitis from healthy and periodontally diseased patients has also been examined. The results showed higher amounts of inflammatory mediators in gingivitis sites compared to healthy sites of normal subjects. GCF from gingivitis sites of adult periodontitis patients showed multiple inflammatory mediators, with elevated IgG levels and lower IgA levels (51). A report by Grbic et al. (7(703,896),(738,911)) demonstrated that IgA levels were significantly increased in GCF from gingivitis sites when compared to that from periodontitis sites, suggesting the potential for IgA to function as a protective factor in this local environment with regard to destructive disease processes. An explanation for this finding was provided by Russell et al. (195), who showed that Fabα fragments blocked IgG complement activation, which has been proposed to contribute to a destructive inflammatory response. Available data also suggest that inflammation of the periodontium, clinically identified as gingivitis, may express an inflammatory profile that is regulated by genotypic characteristics of the patient (69, 125).

The inflammatory cellular infiltrate in gingival tissues and GCF is generally predominated by polymorphonuclear leukocytes; however, B cells and plasma cells comprise a substantial proportion of the mononuclear cell infiltrate in lesions of aggressive periodontitis (e.g. early onset periodontitis). The proportions of infiltrating mononuclear inflammatory cells in granulation tissues from these patients showed that B cells predominated, with lower numbers of T cells (67). These plasma cells are predominantly IgG with lower numbers of IgA cells (139). Extensive studies have been performed examining the

### Table 1. Immune components in the oral cavity

<table>
<thead>
<tr>
<th>Immune system</th>
<th>Inflammatory/innate components</th>
<th>Cellular components</th>
<th>Humoral components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival</td>
<td>prostaglandins, leukotrienes, interleukin-1αβ (IL-1αβ), IL-2, IL-6, IL-8, interferon-γ, colony stimulating factor, tumor necrosis factor-α, acute-phase proteins, transferrin</td>
<td>polymorphonuclear leukocytes, monocytes, T cells, B cells</td>
<td>immunoglobulin G (IgG)/IgA/IgM, C’</td>
</tr>
<tr>
<td>Serum</td>
<td>acute-phase proteins, IL-1β, IL-6, tumor necrosis factor-α, transferrin</td>
<td>polymorphonuclear leukocytes, monocytes, T cells, B cells, natural killer cells</td>
<td>IgG/IgA/IgM, C’</td>
</tr>
<tr>
<td>Saliva</td>
<td>amylase, lysozyme, lactoferrin, cytokines?</td>
<td>Epithelial cells</td>
<td>SlgA/IgG, mucins, PRP3, histatins, defensins</td>
</tr>
</tbody>
</table>

### Table 2. Composition of gingival crevice fluid

<table>
<thead>
<tr>
<th>Cellular</th>
<th>Epithelial – desquamation (oral, sulcular, junctional)</th>
<th>Leukocytes – from systemic circulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GCF</td>
<td>Peripheral blood</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>95–97%</td>
<td>60%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2–3%</td>
<td>5–10%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1–2%</td>
<td>20–30%</td>
</tr>
<tr>
<td>T Cells</td>
<td>29%</td>
<td>50–75%</td>
</tr>
<tr>
<td>B Cells</td>
<td>71%</td>
<td>15–30%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein/Soluble</th>
<th>IgG1 to IgG4; complement components</th>
<th>Prealbumin, albumin, fibrinogen, ceruloplasmin, transferrin, haptoglobin, hemopexin, β-lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines, chemokines, prostanoids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Evidence for local production of immunoglobulins in GCF

Advanced clinical stages of periodontal lesion are populated by a large proportion of B lymphocytes and plasma cells (191, 203). Numerous investigations have shown that IgG plasma cells predominate in these periodontal tissues (139, 172), which is reflected in IgG being the primary immunoglobulin in GCF (96, 207). Early studies by Mackler and others (139, 141, 235) described the subclass distribution of the IgG plasmacytes and suggested an enrichment for IgG4 cells in the diseased tissues. However, these studies did not differentiate between disease classifications, possible variations in the biofilm composition, or stage of disease progression. More recent studies have identified proportions of IgG cells in the gingival tissues and noted IgG1 > IgG2 > IgG3 ≥ IgG4 and IgA1 with high IgA2 cells observed in advanced lesions (67). Kinane and co-workers (218) demonstrated that IgG, IgA, and J-chains were locally produced in periodontitis tissues. They also noted that IgG1 was the predominant IgG-expressing plasma cell in gingiva and granulation tissues. In contrast, IgA was primarily expressed in the gingiva, with IgA1 predominating, although IgA2- and J-chain-positive cells were also enriched in the gingiva. Interestingly, deeper tissue was noted to contain IgM plasma cells, with lower levels of other isotypes. The results suggested that this microenvironment presented features of both mucosal and systemic immune responses. Recently, a large number of B-lineage cells in gingival tissues were observed to present a phenotype that can strongly stimulate autoreactive T cells (201). Specifically, these B-1 cells have been identified as having the capacity to produce polyreactive antibody and appear to be elevated in inflamed gingival tissues compared to peripheral blood, suggesting a contribution to early host defenses in this local environment (173). Thus, immunoregulation of local polyclonal B-cell activation has been suggested to contribute to the progression of periodontitis (192, 224).

Investigations of GCF immune responses have described the levels and isotypes of local antibody. These studies have demonstrated elevations in immunoglobulin in gingival tissues (120, 207, 214) and GCF (96, 193, 200), which were consistent with a local synthesis of these molecules. Certain of these studies have also demonstrated the presence of specific antibody activity to suspected periodontopathogens in the immunoglobulin composition of GCF (11, 43, 57–59, 74, 207, 229). Generally, these results demonstrated localized elevations in specific antibody in GCF (11, 57, 59, 207) (Fig. 1). As such, a proportion of GCF samples with elevated antibody frequently harbor the homologous bacteria, as well as suggesting that a combination of the antigen and the host-response is frequently associated with progressing disease (57) (Fig. 2). However, we still have little information on the functional importance of this GCF antibody, somewhat in contrast to a variety of in vitro and in vivo studies describing the capacity of serum antibody to interfere with various virulence properties of oral pathogens (Table 3). In addition, there are four subclasses within the IgG isotype of immunoglobulin, IgG1 to IgG4, which have been shown to be elicited by different types of antigens and to exhibit diverse functional capabilities (89, 140). Some early reports described the distribution of IgG subclass plasmacytes within the periodontium and subclass levels in serum (49, 141) and suggested that the IgG4 subclass was expressed in proportions disparate from serum concentrations. The presence of all subclasses of IgG have been identified in GCF, with IgG1 and/or IgG4 levels in GCF elevated relative to serum concentrations (59, 140). These results were extended in a study, in which Powell et al. (189) showed the presence of all subclasses in GCF although, the IgG4 subclass was elevated relative to serum concentrations in adult periodontitis. A previous study by Reinhardt et al. (193) indicated that
Fig. 1. Distribution of antibody in gingival crevice fluid (GCF) to *Actinobacillus actinomycetemcomitans* in aggressive (AP) and chronic (CP) periodontitis patients, all of whom had documented oral infections with this pathogen. Each bar denotes an individual patient and represents the frequency of GCF samples with immunoglobulin G (IgG) antibody significantly elevated when compared to a matching serum sample from the same individual. The data describe extensive variability in the frequency of GCF samples with evidence for local antibody production, irrespective of the clinical classification of the patient.

Levels of GCF IgG1 and IgG4 were significantly elevated in active when compared to stable sites in periodontitis patients and that IgG1 and IgG4 levels in GCF were elevated relative to serum. In particular, IgG4 in active sites was nearly 25 times the serum level. Similarly, IgG1 and IgG4 were found to be the primary IgG subclasses synthesized by tissue derived from periodontitis sites (214). A more recent study investigated total IgG subclass levels in periodontitis patients and matched controls (38), reporting that...
IgG2 levels were significantly higher in patients than in controls, and suggesting that the predominant antibody response to periodontal pathogens is directed against carbohydrate or glycolipid antigens. Thus, the literature provides rather compelling results supporting a local synthesis of IgG in the periodontium.

Finally, Mallison et al. (143–145) examined specific antigens and nonspecific immune activators in chronic inflammation, as well as the effect of the chronic inflammation itself on local gingival responses. They noted that accumulation of plasma cells in inflamed sites is promoted by chronic inflammation, activators of microbial origin, and specific antigen. This group also examined the potential of polyclonal B-cell activators from periodontitis-associated bacteria to contribute to the local immunoglobulin repertoire. They reported that compared with resting B cells, recently stimulated B cells clearly differentiated more readily into antibody-forming cells. Thus, antibody synthesis specific for nonoral antigens did occur in inflamed gingival tissue, and a number of mechanisms, including polyclonal B-cell activators, probably contributed to this phenomenon. Generally, they concluded that this milieu can be expected to develop in some periodontal lesions and could help explain why GCF from some sites may contain extraordinary levels of locally produced specific antibodies for certain antigens.

### Systemic contribution to immunoglobulins in GCF

An intriguing aspect of humoral responses in periodontitis has been the relationship between local and systemic antibodies. The general paradigm existed that GCF was comprised primarily of a serum transudate at the site of inflammation (20, 24), and that as inflammation progressed or expanded, the transudate would increase, thus contributing to elevations in local immune mediators. However, it has been suggested that serum antibody specificities may reflect a local gingival response to oral microorganisms, such as *A. actinomycetemcomitans* in periodontitis patients (42, 45, 56). This concept is consistent with recent evidence suggesting the presence of dendritic cells that could sample antigen through mucosal tissues and migrate to the local draining lymph nodes as a long-lived source of antigen presentation related to host–parasite interactions in the local (e.g. gingival) environment (130, 159). As such, serum IgG antibody responses to outer membrane proteins, capsular polysaccharides, high molecular weight carbohydrate antigens, and lipopolysaccharide have been demonstrated to periodontal pathogens that may have been triggered and matured within the milieu of the gingival tissues (16, 19, 55, 185, 206, 236, 237).

We have identified a positive correlation between IgG3, IgG4, and IgG2 levels in serum and GCF samples demonstrating elevated antibody in each of these subclasses. We interpreted these findings as consistent with systemic antibody being a manifestation of the local antibody responses in the gingival tissues, and that a portion of serum antibody may be derived from local gingival responses to the infection. In contrast, IgG1 showed a very low correlation and could suggest different mechanisms of antigen processing and presentation for the synthesis of this subclass between the local and systemic tissues. The antigenic specificity of GCF antibody has generally been estimated using whole microorganisms or complex sonicated antigen preparations (11, 58, 74, 229). We used Western immunoblotting to define more critically the antigenic specificity of GCF antibody to outer membrane components from *A. actinomycetemcomitans* (47). The results showed that many of the GCF samples from periodontitis patients showed an identical antigen reactivity pattern to those observed with the homologous serum. Notably, a proportion of the GCF samples were reactive with antigen(s) that were distinct from those detected by serum antibody specificities. This finding provided evidence for a local stimulation of antibody-producing cells that may be under local immunoregulation and antigen presentation that was limited to the immunologic components in the gingival tissues. Moreover, this finding supported the
possibility that the serum antibody specificities may reflect gingival tissue antigen processing, resulting in both a local and a systemic response. Evidence from various studies have indicated migration of immune cells to the inflammatory lesion in the periodontium (143, 204), but minimal information is available which shows a migration of immune cells from the gingiva into the periphery. To understand best and utilize the functional capabilities of the local and systemic immune responses, we will need to clarify these characteristics of the host–parasite interactions.

Complement activation and function within GCF

Complement activation is generally considered to be a protective mechanism in antibacterial immunity, although, products of this pathway clearly have pro-inflammatory activities that can lead to tissue destruction. Numerous complement components have been reported in GCF, derived from serum and/or local synthesis. Even in a healthy gingival sulcus there is a biofilm of bacteria, and the GCF fluid transudate contains both C3 and C4. However, conversion of C3 is minimal, presumably reflecting the fact that the intact epithelium is an effective barrier or that the bacteria associated with the biofilm at healthy sites are not particularly active in stimulating alternative complement fixation (121). Cleaved C3, indicating the conversion of this molecule presumably through the alternative pathway, is increased in GCF at gingivitis sites (5, 186). Similarly, only C3 is activated in the gingival sulcus of chronic periodontitis patients (154, 163, 164), albeit at somewhat higher levels than in gingivitis. In aggressive periodontitis (e.g. early onset periodontitis), activation products, including C4a, C3b and C5a, are found indicating activation of both the classical and alternative complement pathways (198) and emphasizing the potential of antigen–antibody complexes contributing to local immune modulation. Additional studies have attempted to expand the potential biological importance of complement within the periodontium. Recent work has suggested that degradation of complement components by Porphyromonas gingivalis gingipains may play a key role in subverting host responses (174, 197). In contrast, antibody to a major outer membrane protein of P. gingivalis effectively killed the microorganism by activating both classical and alternative complement pathways (210). Significant amounts of bone-resorbing IL-6 are released by osteoblast-like cells stimulated by C5a (188), suggesting a potential tissue destructive contribution from this type of host response.

GCF antibody

A breadth of information on humoral immune responses in periodontitis has emphasized systemic antibody characteristics. However, it is clear that the local periodontal tissues manifest a broad array of functional immune cells including antigen-presenting cells, regulatory T-cell phenotypes, B cells, and plasmacytes. These findings resulted in continual reports attempting to characterize the local gingival humoral response, as well as relating this response to the specificity, levels, induction, and disease association of local antibody to oral infections. Advanced clinical stages of the periodontal lesion are populated by a large proportion of B lymphocytes and IgG plasma cells (68, 139) that are reflected in IgG as the primary antibody component in GCF (57, 59, 207). Thus, the immunoglobulins being produced by plasma cells at the tissue site, their subclass distribution and relationship to immunoglobulins detected in the GCF are of relevance to local and systemic immunity. It is also clear that these B lymphocytes are proliferating and differentiating in an environment of antigen-presenting capabilities and T-cell regulatory influences. Seymour et al. (202) have proposed a model of the immunopathogenesis of chronic inflammatory periodontal disease. Their model suggests that susceptible subjects may have an increase in T helper type 2 cells selectively homing to the gingiva and leading to B-cell expansion. This process may trigger periodontal destruction via local IL-1 release or protection via production of specific antibody by antigen-specific B cells. Resistant subjects were proposed to exhibit an increase in T helper type 1 cells selectively homing to the gingiva resulting in B-cell suppression and a stable T-cell lesion with slow tissue destruction. In this regard, polymerase chain reaction amplification of the V-J junctions of the T-cell receptor-γ gene rearrangements showed that T cells localizing in gingiva differ from those in skin and peripheral blood (113). They concluded that these findings are consistent with the existence of local immune systems, one of which was composed of gingiva-homing memory T cells. These models suggest a unique immunological micro-
environment in the gingival tissues that can regulate the resulting characteristics of the B-cell responses and orient them towards maintenance of health or progressing tissue destruction. This concept has been extended by Cutler and colleagues (105) who showed that in chronic periodontitis tissues, CD1a+ immature Langerhans cells infiltrate the gingival epithelium, whereas CD83+ mature dendritic cells specifically infiltrated the CD4+ lymphoid-rich lamina propria. Their findings suggested a mechanism for the pathophysiology of periodontitis involving the activation and maturation of dendritic cells in oral tissues, leading to a local release of specific cytokines and the formation of T cell–dendritic cell foci. They have suggested that these foci present some characteristics of oral lymphoid follicles that could provide similar functions to Peyer's patches.

Gingival health is characterized by an absence of an inflammatory infiltrate; although, histologically, gingival health usually represents a balance between the existing subgingival microbiota and host resistance factors. There is some minimal inflammation with an associated flow of fluid into the healthy sulcus and the existence of some inflammatory cells in the tissues. In this state, the gingival tissues contain low numbers of leukocytes that are primarily classified as T lymphocytes (135, 203). While some inflammatory cells and mediators are detectable, low levels of products of the humoral immune response have been identified at healthy sites. Those antibody molecules that are detectable, are quite low, and most frequently react with organisms from the Gram-positive microbiota commonly associated with early plaque development. Thus, even in clinically healthy sites, it appears that the host controls the local response capabilities and suggests some intrinsic homeostatic control of the inflammatory response (54). We have examined mediator profiles in clinically healthy sites from normal subjects, as well as from patients with periodontitis or following treatment (adult maintenance). These results generally showed higher IgA levels associated with health.

Gingivitis is primarily a nonspecific inflammatory response to the bacteria in plaque. It includes a vascular response with increased fluid accumulation and inflammatory cell infiltration. The early response is mostly lymphocytic represented by T cells; however, advanced and more chronic gingivitis can contain plasma cells. These local tissue responses are not associated with bacteria in tissues, but are in response to products that appear to cross the gingival epithelium, which has lost some of its innate protective barrier functions. The predominant cell type in inflamed gingiva from adolescents has also been identified as T cells (14). In experimental gingivitis, the inflammatory lesion is comprised of lymphocytes (112), with total T cells and polymorphonuclear cell levels peaking early in experimental gingivitis and then decreasing, which would be consistent with the B-cell infiltrate noted in more advanced periodontitis tissues (101, 191). In experimental gingivitis it was recently reported that T-cell receptor Vβ genes are expressed in both healthy tissues and peripheral blood. However, a restricted expression of the Vβ repertoire was suggested during gingivitis similar to that described in chronic periodontitis (106). Examination of local inflammatory responses in experimental gingivitis has also demonstrated some differences between young and old subjects. Variations in acute-phase reactants and some immunoglobulins were noted, as well as an increase in B cells and a decrease in polymorphonuclear leukocytes in the older subjects, implying some changes in the characteristics of the host response to plaque associated with aging (112). IgG antibodies to *Actinomyces* spp. were found to be higher in gingivitis (56), and a similar study showed that antibodies to at least three bacteria (*Actinomyces* spp., *Bacterionema matruchotti*, *Leptotrichia buccalis*) were detected in gingivitis patients with a wide variation in levels (56). Multiple reports have suggested that gingivitis patients can present with antibody to suspected oral pathogens (42, 45, 56) including *P.gingivalis* and *A. actinomycetemcomitans*. Bimstein & Ebersole (15) noted that IgM antibody levels were significantly different to many bacteria when comparing children and adults with gingivitis. In contrast, the IgG levels were unrelated to disease in the adult groups, but did help to distinguish between children with and without gingivitis. Thus, generally, the gingivitis patients exhibit antibody to a wide array of these bacteria; however, the levels to suspected periodontopathogens are uniformly significantly lower than those seen in periodontitis patients (31, 56).

The literature is reasonably consistent in suggesting a lack of IL-4 in GCF or cells in gingival tissues of chronic periodontitis patients (67, 118, 139, 146, 234). However, a recent report by Seymour et al. (202) determined that subjects susceptible to periodontal breakdown have an increase in IL-4-producing T helper type 2 cells and nonsusceptible subjects exhibit an enrichment of IL-2/interferon-γ-producing T helper type 1 cells in the gingiva. In contrast, high levels of IL-5-producing cells have been detected in chronic periodontitis gingival
tissues (68, 234). Since B lymphocytes and plasma cells have been identified as significant components of the cellular infiltrate in progressing periodontitis, numerous studies have also examined IL-6 production in these tissues as a regulator of B-cell expansion (126). These studies have shown the following: IL-6-producing cells elevated in inflamed chronic periodontitis gingival tissues (68, 71, 116, 219, 234); GCF IL-6 levels correlated with bleeding, pocket depth, and disease active sites (71); IL-6 mRNA detected in both lymphoid and nonlymphoid cells of inflamed tissues (219); and local B-cell differentiation into plasma cells correlated with high local IL-6 levels (118). Recently, regulatory aspects of the cells in the gingival tissues have shown that immunoglobulin production by gingival mononuclear cells is suppressed after in vitro stimulation (136), but can be increased after removal of CD8+ cells (222). These results suggested that B cells in the gingival tissues are already stimulated, which is consistent with elevated IgG levels in GCF from chronic periodontitis patients with active disease (57, 58, 122, 207, 238). Furthermore, IgG1 and IgG4 (181, 189, 238) appear to be specifically elevated in GCF in active sites of chronic periodontitis patients. Soluble Fcγ-binding components, e.g. FcγRIII, have been detected in GCF from periodontal lesions (242). The results showed elevated GCF levels of this molecule when compared to matched sera, suggesting a potential contribution to disrupting local homeostasis. IgA levels have been routinely negatively correlated with attachment level, pocket depth, and bleeding before and after treatment (77, 121). Since, IgA synthesis is highly dependent on T helper cells, the lower number of these regulatory cells in destructive disease would provide a link in T-cell–B-cell progression in the disease.

Various studies have examined specific antibody responses in GCF from chronic periodontitis patients. Baranowska et al. (7) found no significant difference in the level of specific IgG to P. gingivalis in GCF between healthy and diseased sites within the same individual. Similarly, Tew et al. (229) found no obvious differences in the clinical parameters of probeable pocket depth and attachment level between sites with elevated antibody to P. gingivalis and/or A. actinomycetemcomitans, and those with normal or low levels, and concluded that elevated antibody in GCF may relate to changes in disease activity that are not detectable by normal clinical assessments. Suzuki et al. (216) demonstrated that local production of IgG to P. gingivalis was markedly increased in chronic periodontitis, suggesting that disease progression is influenced by local antibody production. Challacombe et al. (21) showed that levels of IgG antibody to P. gingivalis were lower in the GCF of patients with a high periodontal disease index, although this was not found to be statistically significant. Interestingly, opsonic activity in the GCF against P. gingivalis was found to be significantly depressed with increasing periodontal disease. Lamster et al. (123) reported a significant correlation between total IgG in GCF and specific serum antibody to Prevotella intermedia but not to P. gingivalis. These findings suggested that a local deficiency of IgG to oral pathogens may lead to local disease progression. In contrast, Killian (110) has demonstrated that P. gingivalis can degrade human IgG and IgA, suggesting that low GCF levels of IgG may be caused by degradation by this microorganism, or that locally available antibodies are adsorbed by the greater mass of subgingival plaque present. Jansen et al. (99) have reported further evidence showing the ability of P. gingivalis and other oral bacteria to degrade IgG, which may be an important contributor to the virulence of polymicrobial infections. Nevertheless, a significant frequency of GCF samples with elevated antibody to P. gingivalis in sites of periodontitis from chronic periodontitis patients compared to normal sites has been reported (7, 26, 216). Thus, because elevations in local GCF antibody (7, 26, 58, 114) and gingival tissue responses (171, 172) to P. gingivalis have been identified, this microorganism does provide antigenic stimulation at sites of periodontal disease. Ogawa et al. (171) showed an increased proportion of IgG3 and IgG4 at local periodontitis sites in response to P. gingivalis fimbriae. Longitudinal studies have suggested that P. gingivalis responses are unique in certain individuals and that these levels modulate with episodes of disease activity and treatment (22, 115, 196). Various other investigations have also shown that treatment of periodontitis will often result in decreases of systemic antibody to P. gingivalis after an interval (22, 60, 98, 196). It has been previously demonstrated that lower GCF antibody levels to P. gingivalis were found in deeper pockets and in more inflamed sites (114), and this has recently been confirmed in a cross-sectional study of healthy, gingivitis, and periodontitis sites in patients with periodontitis (156). This study showed that periodontitis sites have lower antibody levels to P. gingivalis than gingivitis sites within the same patient. OuYang (178) substantiated this finding, showing that periodontitis...
patients had lower GCF levels of antibody to *P. gingivalis*, when related to serum levels, than gingivitis patients. These authors had previously demonstrated that elevations in the GCF:serum ratio of antibody to *P. gingivalis* occurred 1 month after periodontal treatment (177). It appears that local antibody consumption may be reduced after removal of the organism and that the GCF:serum ratio of antibody level might be used as a significant indicator in the evaluation of treatment effectiveness. Moreover, it has also been established, that IgG levels are lower in inflamed tooth and implant sites than in healthy matched sites (1). Finally, anti-desmosomal IgG antibody was higher in chronic periodontitis GCF compared to GCF from control patients, and these levels were higher in diseased sites vs. healthy sites from the same patients (76), suggesting the potential for destructive immune responses in the local environment. Immune complex levels have been reported to be higher in GCF from deeper pockets in chronic periodontitis patients (233).

Aggressive periodontitis identifies a group of diseases previously denoted as localized and generalized prepubertal periodontitis, localized and generalized juvenile periodontitis, and rapidly progressive periodontitis. These diseases appear to occur in a ‘high-risk’ group (102) and their hallmark is periodontal destruction that initiates at an early age and of which the rapidity of progression is often not commensurate with the level of local inciting factors. Thus, the immune response characteristics of this group are relatively heterogeneous; however, a few ‘typical’ characteristics can be discerned from the literature.

A proportion of GCF samples within a given subject have local antibody levels significantly greater than can be accounted for by a serum contribution (57, 58, 73, 229). Johnson et al. (103) found GCF antibody levels that were often elevated to *P. gingivalis* in aggressive periodontitis (early onset periodontitis) patients, although the site levels varied substantially within the same patient. Use of gingival explant cultures to examine local humoral responses showed nearly 50% of these cultures from juvenile and rapidly progressive periodontitis (e.g. aggressive periodontitis), 40% of chronic periodontitis, and only 17% of healthy tissue made antibody to *A. actinomycetemcomitans* (87). Additionally, juvenile periodontitis explant cultures produced IgG antibody most frequently to *Eubacterium nodatum* and *A. actinomycetemcomitans*, although more cultures were positive for anti-*P. intermedia*, *Capnocytophaga ochracea*, and *Pepstrepococcus micros* antibodies than healthy tissues, suggesting the potential for multiple bacteria contributing to aggressive periodontitis (88). Ebersole et al. (43) have identified antibody isotypes/subclass proportions in GCF to help clarify the potential protective ability of these antibodies towards infection with *A. actinomycetemcomitans*. The elevated IgG GCF antibodies to *A. actinomycetemcomitans* were noted in 16% of sites and in 90% of the patients, while approximately 15% of the patients had >20% of teeth with elevated IgG antibody to *A. actinomycetemcomitans*. These prevalence data were substantially greater than previous frequencies reported from general periodontitis populations, probably a result of focusing on patients who were all infected with the pathogen (44, 58, 207). We also noted the presence of elevated IgA antibody to *A. actinomycetemcomitans* in approximately 3% of the sites, consistent with previous data reported by Smith et al. (207). However, not a single sample with elevated IgM antibody to *A. actinomycetemcomitans* was observed, out of the >500 sites evaluated. This agreed with previous studies that demonstrated a general lack of local production of IgM, as well as IgM plasmacytes, in the gingival tissues (120, 214, 220).

Variations in the local IgG antibody response to *A. actinomycetemcomitans* were also documented (43). Examination of absolute levels of antibody in the GCF demonstrated all subclasses. Approximately 57% of GCF samples exhibited antibody that was within the range of the homologous serum sample and demonstrated a striking increase in IgG1 relative to the other subclasses. While nearly 30% of the samples showed antibody levels that were below the level in serum, the antibody pattern was unique and suggested a major contribution of IgG2 in sites with this profile. Finally, in GCF samples that showed elevated antibody relative to serum, the most dramatic changes were in the levels of IgG3 and IgG4. Cross-sectional studies have suggested that those GCF samples with elevated antibody frequently harbor the homologous bacteria, as well as suggesting that a combination of the antigen and the host-response is frequently associated with progressing disease (57). The majority of sites with elevated IgG3 and IgG4 antibody were also colonized by the micro-organism. For example, >95% of sites with elevated IgG4 were colonized by the bacterium, while <50% of sites with elevated IgG2 demonstrated this micro-organism. We also observed that healthy sites did not contain elevated IgG4 antibody to *A. actinomycetemcomitans* (43), and IgG4 was a principal response at teeth defined as diseased, suggesting a character-
istica of this response at disease sites. IgG2 was most frequently elevated in GCF derived from teeth clinically categorized as diseased. Thus, it could be suggested that the sites with elevated IgG2 may be indicative of areas in which the host response has stabilized a previously active phase of disease. The frequency and distribution of antibody in the GCF, as related to colonization with this *A. actinomycetemcomitans*, were consistent with localized host–parasite interactions at individual tooth sites. These results suggest the potential for this local antibody to *A. actinomycetemcomitans* to play an important role in the gingival sulcus in relationship to colonization and clinical presentation. There also appears to be a complex association between the distribution of *A. actinomycetemcomitans* infection (i.e. number of teeth infected and proportion of *A. actinomycetemcomitans* per tooth) and the antibody levels to the intact bacteria in GCF (43), supporting a unique local response in individual sites within certain patients and consistent with a progression of subclass responses at sites of infection and disease.

We have extended these studies using both cross-sectional and longitudinal GCF samples to determine the antigenic specificity of GCF antibody. A proportion of GCF samples that exhibited significantly elevated antibody to *A. actinomycetemcomitans* were also examined using Western immunoblotting to outer membrane antigens from *A. actinomycetemcomitans*. Homologous sera were also examined to compare antibody specificities. Of the sites with elevated IgG antibody, 87% were colonized by *A. actinomycetemcomitans*; however, 46% of sites with *A. actinomycetemcomitans* infection did not have elevated antibody. Cross-sectional studies identified a 78–100% agreement between the antibody specificities in GCF and serum. Additionally, patterns of antibody reactivity in GCF and serum in the subjects were often very distinctive. Longitudinal alterations in GCF antibody were examined in 15 patients through a monitoring interval of up to 2 years and showed a general conservation of specificities. However, seven of 15 patients exhibited a definite acquisition of different antibody specificities during the monitoring. These results indicated that the antibody specificities in serum appear generally to reflect the local response to this pathogen (47).

While local humoral immune responses in aggressive periodontitis have generally emphasized infections with *A. actinomycetemcomitans*, studies of GCF from rapidly progressive periodontitis patients showed that sites infected with *P. gingivalis* had high levels of specific GCF antibody to this microorganism, which was related to clinical status. After treatment, these local antibody levels decreased with pocket depth, the attachment level stabilized, and inflammation resolved (102). A compilation of the available research evidence clearly demonstrates a local gingival immune response to various microorganisms. With respect to *A. actinomycetemcomitans* and *P. gingivalis*, this response appears specific, related to the colonization of the site, and can apparently be modified by local therapy, which presumably reduces the antigenic burden. At the same time, it is clear that a serum antibody response exists to these localized infections, and that there is some relationship between the local and systemic response levels and antigenic specificities. However, it is still unclear as to how the derived immune components complement or antagonize to afford some protective capacity within the infected gingival sulcus.

**Medically/environmentally compromised patients**

Generally, there are minimal data regarding local immune responses in compromised patients. In autoimmune diseases such as Sjögren’s syndrome there is an immune dysfunction and a significant decrease in salivary gland function, resulting in decreased saliva to bathe the oral cavity (220). Periodontal disease studies in these individuals have not been numerous and the results are varied. Moreover, these studies are frequently complicated by the chronic administration of various anti-inflammatory and immunosuppressive agents as palliative measures. We have completed a study of patients to describe the microbiology and local and systemic host responses associated with periodontitis in these patients. Sjögren’s syndrome patients exhibited a higher plaque index, higher decayed, missing, filled surfaces (DMFS) score, increased attachment loss, and increased destruction of alveolar bone, when compared to matched controls that were accompanied by elevated antibody to *A. actinomycetemcomitans* and/or *P. gingivalis*. These results suggested that common periodontal pathogens are associated with disease in Sjögren’s syndrome patients, and that the oral complications of Sjögren’s syndrome may alter the characteristics of the bacterial colonization related to periodontitis (212). We also examined GCF for various inflammatory mediators and levels of IgG and IgA. The results demonstrated that GCF fluid volumes re-
lated to local inflammation were similar in Sjögren's syndrome and control patients. Differences were noted in levels of various inflammatory mediators, with higher IgA levels in GCF from the Sjögren's syndrome patients (211). These findings supported some contribution to gingival immune responses by the alterations associated with this autoimmune disease.

Prominent risk factors or risk predictors for periodontitis in adults include smoking, diabetes, race, low education, infrequent dental attendance, and genetic influences. Several specific bacteria or microbial consortia have been shown to be associated with loss of periodontal support and are considered to be risk indicators of disease. Clinical signs of periodontal disease, such as pocket depth, loss of clinical attachment and bone loss, are cumulative measures of past disease. Of the systemic diseases that have been related to periodontitis susceptibility, diabetes mellitus, has provided the strongest link. Both type 1 and type 2 diabetes clearly increase the risk for and severity of periodontal destruction. While some variations in microbial challenge in these patients have been identified, the breadth of information suggests a host-controlled responsiveness, or lack thereof, leading to more severe disease. Diabetic patients have altered inflammatory responses, neutrophil dysfunctions, wound-healing capacity, and local cell functions to maintain homeostasis (28, 194). A few reports have identified altered serum antibody to oral microorganisms in diabetics (52, 158, 243). However, while studies have reported variations in GCF and gingival tissue mediators, there are negligible data on local humoral responses. This would appear to be an area for research to focus on better understanding this oral–systemic disease linkage.

Almost every report available has documented a relationship between smoking and periodontal disease. Tobacco smoking is probably the most important, controllable environmental risk factor in periodontitis (83, 200). The effect of smoking on gingivitis appears to be a function of the amount of local plaque challenge, as well as a measure of smoking exposure. In almost all populations studied there was a significantly increased frequency of periodontitis in patients who smoked (72). Smoking was strongly correlated with severe bone destruction in aggressive periodontitis, particularly in generalized disease patients (72, 190). Bergström & Preber (12) provided data indicating that smokers vs. non-smokers exhibit similar periodontopathogens, thus supporting a role for altered host responses in susceptibility to increased disease. We reported a study examining aggressive periodontitis smokers compared to healthy nonsmokers (HNS) and smokers with only gingivitis (HS). We noted that total IgG and IgA in GCF from aggressive periodontitis patients were significantly elevated compared to both HNS and HS, while IgA was significantly decreased in the HS group. These findings suggested that smoking has a negative impact on the immune system (148) and contributes to local host immune alterations and susceptibility to colonization/infection with certain pathogens in patients. However, more detailed information regarding the ability of the local tissues to mount specific humoral responses, as modified by smoking remain to be gathered and evaluated.

Clinical implications of local humoral immune responses

Periodontitis is a disease of bacterial etiology and the progression of disease may be related to both direct bacterial effects on host tissues and activation of a large complex of autocrine and paracrine factors that can amplify local inflammatory reactions with resulting tissue damage. The recognition that all individuals are not equally susceptible to periodontitis and that the quality and quantity of the subgingival microbiota can vary among individuals has led to a focus of investigations examining possible diagnostic tools in the management of periodontitis. The concept that an immunologic test could be useful both as a predictor of disease (and its activity), as well as in the diagnosis and treatment of periodontal disease have a solid foundation, because these types of tests are currently used in many infectious diseases. A recognition of the various questions that need to be addressed for better understanding of the disease, especially its initiation and progression, as well as treatment and prevention indicates that immunologic tests could be designed with different goals. Currently the characteristics of periodontitis (i.e. classification, prognosis, treatment success) are generally subjectively determined. The wealth of data, with the above as examples, is consistent with antibodies being a reflection of the host response to an infectious process associated with an episode of disease activity and potential usefulness in disease diagnosis/categorization. The use of immunologic tests in the diagnosis of periodontitis should provide the ability to examine objectively numerous aspects of the disease process.
Etiology and directed treatment information

While many of the periodontal pathogens can colonize other niches in the oral cavity, a primary site of infection is in the biofilm of the subgingival ecology. The host recognition of these bacteria that leads to adaptive antibody may vary and the findings of differences in isotype and subclass responses suggest that:

- the variation is in response to different types of dominant antigens stimulating the host, or
- the variation is a result of differences in ecologic niche and/or tissue interactions of the different bacteria with the host.

Evidence describing a microbial progression in the subgingival microbial ecology associated with periodontitis has supported the potential for microbiological discrimination of periodontal risk (41, 221). Socransky and colleagues (84, 209) summarized these microbiological research findings and suggested that evaluation of these etiologic agents may contribute to earlier diagnosis, and both earlier and better targeted intervention strategies. Genco et al. (73) have also defined microbial pathogens with respect to their origin and suggested elucidation of the infection as a method to treat periodontitis as an infectious process. The process of utilizing antibody responses to detect diseases caused by specific infectious agents is widespread in medically important infections. The use of immunologically based diagnostic procedures is generally associated with those diseases in which the infectious agent is difficult to obtain in samples or culture in the laboratory. Diagnoses based upon host antibody responses have been used to delineate the course of an infection by:

- differentiating between specific agents that induce similar symptoms;
- assessment of the onset of an infection;
- determination of remission from an infection; and
- defining the success of directed treatment regimens.

The humoral components of GCF have also been suggested as factors for monitoring periodontitis (57, 73). In particular, inflammatory and immune mediators in GCF change coincident with clinical measures of gingival inflammation and progressing periodontitis (54, 123, 170) and presumably contribute to this process. Since these host responses presumably arise by microbial species or their products entering the periodontal tissues and eliciting an enhanced host inflammatory/immune response (183), the data suggest that immunoglobulins and specific antibodies in GCF may be important contributors to monitoring etiologic agents in this disease.

Diagnostic information

Periodontitis is clearly a multifactorial disease that appears to result from a combination of parameters including genetic susceptibility (102), challenge (e.g. infection) with a specific pathogen (208), and characteristics of the host inflammatory/immune response (179, 191). Current thoughts on host–parasite interactions in periodontal disease(s) emphasize a specific bacterial etiology, rather than a nonspecific incitement directly related to plaque mass, albeit, the concepts of ‘microbial consortia’ and ‘biofilms’ as more representative of the pathogenic plaque environment have generated substantial support in the etiology of these diseases (73, 183, 209). Variations in onset, severity and clinical characteristics support the existence of different forms of periodontitis, which may have different microbial characteristics. These findings have specifically recognized and described various forms of periodontitis including: localized juvenile, rapidly progressive, chronic adult, refractory, AIDS-associated, and diabetes mellitus-associated; that may have unique etiologic agents (183), although the recent World Workshop in Periodontal Disease, reclassified these diseases based upon rapidity and extent of the disease process, response to therapy, and other modifying factors (4). Original concepts of periodontitis suggested that this disease was a chronic process that was most directly associated with plaque mass in the subgingival sulcus and would progress inexorably with age in the majority of the population without active measures to remove the plaque and its ecologic niche. Changing concepts of this paradigm during the past decades clearly argue that specific component microorganisms within the plaque appear to be a more accurate indicator of disease progression. Epidemiological investigations have also suggested that only a subset of the population expresses susceptibility to severe periodontitis, and prospective longitudinal studies have provided evidence that while periodontitis in some individuals appears to proceed as a chronic progressive destruction, other individuals demonstrate a disease that exists as exacerbations (active) and remission (inactive). The frequency and length of the active episodes and length of intervals of remission may discriminate between the most severely diseased patients, including aggressive and refractory periodontitis (85). Thus, the concept of peri-
odontitis as a disease of exacerbations and remissions, suggesting a dynamic process, has altered experimental designs for the monitoring and treatment of the disease, as well as the host response that may vary during these intervals (8). These concepts also have an impact on the significance of host immune responses for diagnosis of different forms of periodontitis, which may have different microbial characteristics. Studies have been implemented to examine the use of clinical and laboratory parameters for prediction or risk assessment of periodontal disease. Various investigators have proposed the potential to utilize antibodies or local mediators in GCF (54, 57, 74, 170, 223) as adjuncts in the diagnosis of periodontitis and in defining the mechanisms of disease. The studies described in previous sections of this review document some characteristics of the local immune response and their relationship to infection and clinical presentation in periodontitis patients particularly within *A. actinomycetemcomitans*- and *P. gingivalis*-infected periodontitis patients. The findings are consistent with the potential to utilize antibodies or local mediators in GCF as adjuncts in the diagnosis of periodontitis and in defining the mechanisms of disease. It is also feasible that certain subjects have specific subclass responses which are reflected in these studies and which are instrumental in rendering them susceptible or more resistant to the disease.

The humoral immune response has been suggested as a means of differentiating between distinct periodontal disease states. For example, a strong local and systemic antibody response to *A. actinomycetemcomitans* has been consistently identified in localized aggressive disease patients (19, 53, 62, 129). An association between the most severe and extensive cases of generalized periodontitis and the relative lack of antibodies to *P. gingivalis* and/or *A. actinomycetemcomitans* has also been reported (65, 82). An interpretation of these findings would be consistent with an interference of disease progression in localized disease by an effective immune response, while those with an ineffective response would go on to develop the more generalized disease. Thus, diagnostic processes could be based upon a rather simple and sensitive detection of antibody reacting with specific pathogens or groups of pathogens that provide an etiologic basis for disease in individual patients. To address these various questions, different tests may be used, each being designed for optimal acquisition of specific data items. At least three types of diagnostic tests are available for the objective identification of the parameters of a large number of diseases, including periodontitis. Discovery (screening) tests have been designed as rapid, inexpensive tests that can provide an initial positive (or negative) indicator of suspected disease. Exclusionary tests are often used to extend preliminary data from discovery tests and are used to differentiate specific phenomena, which exist among a variety of etiologies. Confirmatory tests are used following differential diagnoses to define specifically the disease or its etiology. Immunological confirmatory tests would be of significant value in longitudinal monitoring of disease activity, because the level of infection by specific bacterial pathogens, and the extent of involvement with these pathogens in a deleterious host–parasite interaction can be monitored and controlled. We still lack sufficiently robust data sets to define clearly the clinical usefulness of these various types of immunologic diagnostics. Further testing will require extensive longitudinal studies, including assessment of success/failure ratios in well-designed and executed clinical trials. Various hypotheses have been proposed as to the function and potential use of these antibody findings to assess and/or predict periodontal disease progression. However, as reviewed by Wilton et al. (239) the majority of these studies have been cross-sectional and retrospective and the data were equivocal for identifying active disease. These authors accurately noted a paucity of prospective longitudinal studies that would most directly evaluate local antibody potential for risk assessment of progressive periodontitis.

A large number of previous reports support the hypothesis that periodontitis is an infectious disease with selected members of the oral microbiota closely associated with progressing periodontitis (157, 208). As in other bacterially mediated infectious diseases, this process would be expected to elicit a specific host antibody response (64). However, while most medically important bacterial diseases are acute infections (40), the relationship between the pathogens and the host in periodontitis appears to be a chronic interaction with unknown factors 'triggering' a progressing disease process related to a member(s) of the indigenous ecology. As such, suggestions have been put forth to use antibody testing to predict or provide a risk evaluation for disease episodes or recurring periodontitis. Longitudinal investigations of host immune responses in periodontal disease have generally been directed towards utilization of these response characteristics to acquire a clearer understanding of the mechanisms resulting in tissue destruction, or to test their correlation with disease activity and utility in assessing treatment outcomes.
previous studies using heterogeneous populations of patients with periodontal diseases have suggested some relationship between antibody levels and disease activity; however, consistent findings have not been provided (51, 56). As described above, studies of periodontal diseases have suggested that lesions of destructive disease may progress with intervals of exacerbation and remission. Thus, longitudinal studies are necessary to begin to provide guidelines to clinicians to identify which patients are ‘at risk’ for additional periodontal breakdown. Studies of humoral antibody responses in periodontitis may thus be designed to utilize characteristics of these antibody responses as diagnostic tools for monitoring disease, or as a means to clarify the mechanism of destruction and protection (42, 56, 74, 239). However, a limited number of prospective longitudinal investigations have been reported concerning this disease (56). Longitudinal human studies have developed evidence for the potential of local and/or systemic antibody responses to be used as indicators of susceptibility for progressing periodontitis, predicting imminent phases of disease activity, or as a prognosis of disease recurrence. The results suggested that the dynamic aspects of the host–parasite interactions occurring in this disease can be measured and may be developed as risk indicators (8). As this area of research continues to develop, studies will be implemented which will elucidate the characteristics of host responses which fulfil the criteria of risk factors for identification of causality of periodontitis as described by Lilienfeld (128). Thus, the definition of these host responses as risk factors in periodontitis will require data which delineate:

- the strength of association of the response with disease;
- the specificity of association of the response with periodontitis;
- a positive relationship of degree of exposure with risk of developing disease;
- the consistency of association between the host response and disease in numerous studies;
- the temporal consistency of the host response risk factor in preceding the disease; and
- the biological plausibility indicating that the association of the host response makes sense relative to the current knowledge of the disease process.

An important component of current research designs in periodontal research is the development of indicators or factors that would contribute to a modeling of risk of periodontitis, as well as potentially predicting active disease in these patients (8, 36, 213). In this regard, various clinical parameters of gingival inflammation and periodontitis have been explored for their utility in characterizing risk (91, 124). Beck (8) has reviewed the development of strategies to develop clinical and laboratory tools as risk indicators or factors to enable modeling of risk assessment in periodontitis. Results have been reported recently which evaluated host antibody responses to elucidate the specific etiologic agents and to be used in modeling the risk for future periodontal disease progression in recurring periodontitis (48). The findings in adult periodontitis patients indicated that elevations in certain antibody specificities are most closely associated with patients exhibiting a risk of disease recurrence. Furthermore, analysis of the frequency of antibody elevations suggested that patients capable of maintaining elevated antibody to these pathogens post-treatment, may be indicative of an individual at less risk. A second investigation addressed questions concerning host–parasite interactions in A. actinomycetemcomitans-associated recurring periodontitis. The results showed distinctive characteristics of local and systemic antibody responses and A. actinomycetemcomitans infection in patients with varying extents of recurrent disease. These longitudinal studies developed evidence for the potential of local and/or systemic antibody responses as indicators of periodontal disease recurrence.

The attributes of the relationship that exists between A. actinomycetemcomitans and infected hosts which results in a stable commensal relationship or can develop into a pathologic host–parasite interaction remain to be defined. It is clear that active host immune responses result from this interaction and data are available which indicate a variation in antibody level, avidity, and specificity patterns, over time, that are related to infection and disease. In summary, the current status of knowledge delineating antibody characteristics in periodontitis and changes associated with disease progression and treatment appears to be dependent upon the patient selection, antibody specificity examined, and the design of the study. Importantly, the use of immunologic diagnostic/prognostic tools in periodontal disease still remains primarily restricted to research laboratories. It is incumbent upon the research laboratories to evaluate the practicality of such tools and how the findings may be utilized to enable the practitioner to manage an individual patient’s disease more effectively.
Disease activity and therapeutic decisions

Few studies have primarily been directed towards the use of antibody levels to identify phases of disease activity related to a specific infection hypothesis. The majority of these have utilized serum antibody levels, specificity, and avidity to provide some ‘predictive potential’ for determining active episodes of disease (56). Examples of these types of investigations include a longitudinal study in Pima Indians that indicated that antigens of *P. gingivalis* and *A. actinomycetemcomitans* could help to discriminate the risk of development of progressing periodontitis (81) and was one of the first studies to suggest that serum antibodies to *P. gingivalis* antigens detected prior to bone loss could predict disease progression. Longitudinal changes in serum antibody levels and specificity accompanied disease exacerbations (46) and decreases in the serum IgG antibody resulted from treatment of multiple presumed infectious foci (50). Furthermore, systemic antibody levels/specificity provided some predictive capacity for active phases of disease (45) by exhibiting an increase in serum antibody that was prior to a number of active disease episodes.

Investigations of treatment effects in patients with aggressive periodontitis showed a decrease in serum antibody to *A. actinomycetemcomitans* in about half of the patients with elevated antibody prior to treatment (60), although these changes often take extended intervals of time to be manifest (48). Again, serum antibody to *P. gingivalis* was used in attempts to correlate changes in the levels and/or avidity with progression and treatment of periodontitis. Serum antibodies to *P. gingivalis* antigens were detected prior to bone loss (231) and while serum antibody levels generally do decrease post-treatment, differences in treatment modalities, and the time intervals for sampling, make conclusions concerning the efficacy of this antibody monitoring process equivocal (6, 132, 160, 161, 230). An investigation by Chen et al. (22) described an increase in antibody avidity to *P. gingivalis* after nonsurgical therapy in severe generalized periodontitis patients. Horibe et al. (98) demonstrated that mean antibody titers to *P. gingivalis* decreased significantly after treatment. Periodontitis patients who were seropositive for *P. gingivalis* at baseline (i.e. titer more than twice the control median) responded better to treatment in terms of clinical improvement and increase in antibody avidity during the course of the periodontal therapy (94, 155). All of these investigations focused on changes in serum antibody related to disease activity and/or treatment outcomes. In one of the few existing studies, Johnson et al. (103) demonstrated elevated GCF antibody to *P. gingivalis* in infected sites of patients with severe generalized periodontitis, with antibody negative sites increasing threefold following therapy, which correlated with positive clinical effects of the therapy. However, it appears that additional studies are required to delineate fully the importance of changes in local antibody that may precede active disease or provide a rapid quantitative measure of the success of therapy.

In conclusion, while longitudinal studies of human periodontitis cannot unequivocally delineate the etiology and mechanisms of progression of periodontal disease, the results available provide some interesting hypotheses:

- elevated local and systemic antibody levels to subgingival species appear to relate to the composition of subgingival microbiota;
- microorganisms identified by elevated local and/or systemic antibody levels are detected in the microbiota of active disease sites;
- the relationship between colonization and specific host responses is consistent with a specific infectious disease process; and
- the host responses may help to elucidate etiologic agents and subsequent risk factors preceding the initiation and progression of periodontal diseases.

Local humoral immune responses: vaccines and the future

Systemic antibody has been shown to function in antibacterial immunity by a variety of mechanisms. These include the ability to aggregate the bacteria, inhibit adherence and colonization, enhance phagocytosis, lyze the bacteria, and detoxify endo- and exotoxins. Examination of these functions in periodontal disease have generally been oriented in two directions:

- studies attempting to substantiate the protective aspects of the antibody by relating the levels or presence of antibody activity to the severity of disease, and
- studies examining the ability of antibodies to inhibit colonization, toxic and other proposed virulence components of the bacteria. Currently, our understanding of GCF antibody
characteristics and functions is one of the weakest in providing knowledge of the mechanisms of disease, disease prevention, or recovery from disease. Detailed studies of GCF antibody variations may allow a clearer perspective on the antigens of interest for interference with the virulence properties of the bacteria and a clarification of the host–parasite interactions which contribute to disease. The differences in subclass responses also suggest a variation in presentation of antigenic components to the host if whole cells vs. soluble components or membrane fragments of the bacteria are contacted and processed, and the resulting antibody could contribute to a quite different host–parasite interaction.

Due to the prevalence of periodontitis in the populations, particularly in developing countries, vaccines for periodontitis would be expected to be administered to large numbers of people. While periodontal disease is not considered life threatening, recent reports demonstrate a link between periodontal disease and systemic sequelae of morbidity and mortality in cardiovascular and other systemic diseases (70), emphasizing the potential broader importance of preventing this oral disease. To limit the transmission and/or intraoral dissemination of periodontopathic bacteria, it would appear advantageous for an effective vaccine to induce immunity at three levels:

- local mucosal secretory IgA,
- local draining lymph nodes, and
- circulating specific T and B cell responses.

The primary infection and pathogenic mechanisms in mucosal infectious processes include activities such as microbial attachment, colonization, tissue/cell invasion, and localized toxin release. Thus, the principal efforts of vaccination against these types of pathogens should be to elicit localized immune responses, including immunologic memory that can prevent these virulence determinants. For the development of a vaccine the target bacteria/antigens need to be identified. Even in a complex ecosystem, such as the oral cavity, species come and go, albeit not on a daily basis but it occurs often enough for the presumption of a minimal significant impact of a single specific immunization on the ecosystem. The more complex an ecosystem, the more likely that niches (meaning more than just physical location) will overlap, so that loss of one species is compensated for, either wholly or in large part, by others. The idea of a vaccine to control oral bacterial infections was developed in the early part of this century. Autogenous vaccines, pure cultures, and mixed stock vaccines have all been employed at various times. The abundance of new knowledge now at our disposal, including identification of likely etiologic agents, a better definition of mechanisms of pathogenesis, and elucidation of the host protective responses, development of strategic vaccines with various biological characteristics for periodontitis, can be considered.

A broad array of vaccine strategies are currently available, including attenuated and inactivated bacterial vaccines, purified antigen (subunit) vaccines, synthetic antigen vaccines, live viral/bacterial vectors, anti-idiotype vaccines, and nucleic acid vaccines. However, the development of vaccines is a complex process that requires substantial resources over a long period of time (215), with development of vaccines taking an estimated 9–10 years. While classic approaches to vaccine development and implementation have used the strategies described above regarding infecting agent, virulence determinants, and adjuvant enhancement of immune responses, the approaches to immunological manipulation in periodontitis are complicated by a significant confounding factor. In patients with periodontitis, subgingival microbial plaque and components, attract and activate the entire range of host defense mechanisms. Plaque is continuously bathed by the flow of GCF, which arises from blood plasma and contains both humoral and cellular aspects of the host response. Nevertheless, the identification of the extensive variation in the ability of individual bacterial species to evade the host responses, together with the existence of the bacteria in a biofilm, contribute to increasing the plaque microbiota’s ability for the long-term survival of the individual microbes (27, 32). Because of their unique structure, biofilms are substantially more resistant to surfactants, antimicrobials, and antibiotics than planktonic microorganisms of the same taxa. Their enhanced resistance to effector mechanisms of host inflammatory and immune responses is equally important. The molecular mechanisms contributing to the resistance of the biofilm microorganisms, are not completely understood, but presumably hinge upon limited access of the host or exogenous bacteriostatic/bactericidal factors to the bacteria. The formation of microcolonies deep within the biofilm, as well as the production and release of membrane vesicles and cell fragments which distract the effector mechanisms probably contribute to the unique features of the biofilm (32). The resistance of the subgingival plaque biofilms to endogenous host defenses has important implications in consideration of vaccine approaches to periodontal therapy. It is
clear that physical removal/disruption of the biofilms is an essential part of the strategy for maintaining periodontal health, and intervening during periodontal destruction. This biofilm disruption procedure will undoubtedly remain a crucial part of the armamentarium of periodontal therapy. Strategies which link this physical approach to biofilm disruption with the targeting of critical species in the 'pathogenic plaque' should have the greatest probability of success.

Finally, a very recent approach for vaccination strategies against oral diseases, uses the concept of passive immunization. In essence, this approach employs preformed antibodies administered to 'at risk' individuals or to individuals during 'at risk' intervals to interfere with microbial pathogenic processes. A vaccination concept was developed using molecular biologic techniques to enable plants to synthesize and assemble antibody molecules, including antigen-binding domains, complete antibodies, and multimeric antibodies, i.e. 'plantibodies' (137). This potential has already been exploited to create secretory antibodies with heavy and light chains, J-chain, and secretory component (127). Ma et al. (138) characterized a secretory IgG antibody produced in transgenic plants. This antibody was more stable and exhibited a higher functional affinity than the native antibody, and provided protection against S. mutans colonization in humans. Passive immunization with a monoclonal antibody (61BG1.3) was also shown to prevent selectively colonization by P. gingivalis in humans (17, 18). The protective antibody recognized the RI protease of P. gingivalis, both a hemagglutinin and an antigen, which is recognized by sera from periodontitis patients (108). Thus, a passive immunization strategy targeting specific epitopes of an adhesion domain may appear to provide a means of preventing infection with P. gingivalis. The facility for these types of passive immune approaches to have an impact on the health of the oral cavity seems apparent, albeit, substantial research will be required to evaluate their general efficacy.

Page (182) has reviewed the literature pertaining to the humoral response and its relevance to treatment response and vaccine development. In this context, the humoral immune response that occurs in patients with various infectious diseases generally arrests the process of the infection. However, this direct antibody-mediated interference does not appear to occur in periodontal disease, because these opportunistic, commensal microorganisms continue to colonize in the presence of a robust immune response, still provides a conundrum with regards to human vaccine development as a means of addressing this problem.

### Systemic importance of local humoral immune response

Periodontal infections are the result of an interaction between a tooth-associated microbial biofilm and the host defenses. The microorganisms form a matrix-enclosed community which confers a degree of protection on the incorporated microorganisms and offers the possibility of metabolic co-operation. This biofilm, if allowed to mature, harbors large numbers of gram-negative anaerobes, producing a variety of substances that can traverse the epithelium and provoke an inflammatory response (32), in addition to the potential translocation of pathogens into the gingival tissues (66). Various inflammatory defense cells are recruited as a result of this microbial challenge, particularly neutrophils. These emigrating cells, in conjunction with other resident cells, respond to the microbial challenge by elaborating various inflammatory mediators including cytokines (e.g. IL-1β) and prostaglandins (e.g. prostaglandin E2; 121, 166, 234). The chronicity of this inflammatory response can result in the production of tissue-destructive molecules such as matrix metalloproteinases. Subsequently, both host connective tissue and bone can be destroyed, with the current emphasis directed towards the tissue destruction seen in periodontitis resulting from host-derived inflammatory mediators (179, 183). The clinical findings in periodontitis have emphasized the local nature of inflammation and tissue destruction within the oral cavity. Pro-inflammatory cytokines and mediators are significantly elevated with gingival inflammation and during the destructive phase of periodontitis (13, 54, 68, 101, 116, 121, 191, 232). The acute-phase response represents an early and highly complex reaction of the organism to a variety of injuries such as bacterial, viral, or parasitic infection, mechanical or thermal trauma, ischemic necrosis, or malignant growth (117). The acute-phase response is a primary defense reaction and therefore provides protection against bacterial products such as endotoxin (116). This systemic response helps to accomplish the same outcomes as the localized inflammatory response, which is designed to contain or destroy infectious or noxious agents, to remove damaged tissue, and to repair the affected tissue or organ. In-
creased levels of acute-phase proteins have also been noted with gingival inflammation, including during experimental gingivitis and periodontitis, reflecting the locally stressed environment (2, 101, 170, 205, 241). While the majority of studies of periodontitis have emphasized the local nature of this host–bacterial interaction in the periodontium and gingival sulcus (121, 179, 191, 202) it also appears that systemic manifestations of this disease are also detected. Due to the chronic bacterial colonization of the supra- and subgingival aspects of the teeth, the juxtaposed gingival tissue often demonstrates some level of localized inflammation (184). As periodontitis ensues, there are alterations in local host inflammatory mediators (121, 202), the initiation of a localized specific host response (43, 58, 114), and finally, a serum antibody response to the bacteria is observed (42, 152). Consequently, these findings would support some ability of the localized inflammation and/or infection to be manifest systemically within the affected host potentially resulting from transient access of oral bacteria to the circulation (95).

The oral cavity, with its multitude of resident microorganisms, that can reach levels in excess of 10^9 colony forming units per mg of plaque, is not a localized closed environment. Several of the resident microbiota can cross epithelial barriers and reside in deeper tissues and organs of the host. The oral cavity is a nidus for a large number of systemic infections, including soft tissue abscesses (e.g. *Capnocytophaga* spp., *A. actinomycetemcomitans*), bacteremias (e.g. *Eikenella corrodens*, viridans streptococci), and bacterial vascular plaques (e.g. subacute bacterial endocarditis). Clearly, microorganisms resident in a periodontal niche can invade and destroy both localized tissues and deeper tissues and organs far from their original focus of infection. The local gingival immune response to these microorganisms is accompanied by a corresponding serum immune response. A schematic describing these various interactions is presented in Fig. 3. Studies within the past decade have associated periodontitis with several important systemic diseases, thus, the characteristics and function of the local gingival immune response to control these opportunistic pathogens may have much broader consequences to the general homeostasis of the affected individual.

Type 1 diabetes mellitus (formerly insulin-dependent) and type 2 diabetes have both been shown to be major risk factors for the development of periodontal disease in certain populations (81, 175, 176). Type 2 diabetes (formerly noninsulin-dependent diabetes mellitus) is a heterogeneous disorder and accounts for about 90% of the diabetic patients in the United States (3). The prevalence of type 2 diabetes is markedly increased among American Indians, African Americans, and Hispanics (86). Patients with type 2 diabetes may demonstrate normal or elevated basal insulin concentrations combined with insulin resistance and diminished tissue sensitivity to insulin that progressively leads to impaired β-cell function (3). Type 2 diabetes is usually diagnosed after the age of 30 years and is frequently observed as part of a multifaceted syndrome that includes obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease (35). Type 2 diabetes has been estimated to affect 40% of siblings with one affected parent and 80% when both parents have type 2 diabetes (107). In earlier studies of the influence of diabetes on periodontal disease progression it is often difficult to discriminate between patients with type 2 diabetes and type 1 diabetes, except on the basis of age of onset, because few clinical data were provided other than hyperglycemia (109). Studies from Finland have indicated that patients with poorly controlled type 1 and type 2 diabetes demonstrated more gingival bleeding (63), had more periodontal pockets (227), and an increased prevalence and severity of periodontitis and calculus formation (228). The investigators concluded that duration and quality of diabetic control combined with the presence of calculus at periodontal sites were important risk factors for periodontal disease in diabetics. Analysis of clinical periodontal data from the Pima Indian study (162, 199) demonstrated that there was an increased prevalence of periodontal destruction in the diabetic group and that this had led to a greater degree of tooth loss in this population. Further analysis of the relationships between type 2 diabetes and oral health status in this population (61) revealed that only diabetic status, age, and the presence of subgingival calculus were significantly associated with increased severity and prevalence of periodontal destruction, with diabetes increasing the risk of developing destructive periodontal disease about threefold. Taylor et al. (225), examining this Native American population, reported an odds ratio of over 4 for alveolar bone loss in type 2 diabetes. Finally, these same authors noted a significantly increased risk for alveolar bone loss and severe periodontitis progression related to poor glycemic control in these type 2 diabetes (226). The higher prevalence of periodontal destruction in diabetics was also confirmed by Grossi (78) in a primarily non-Hispanic population. In that study age, diabetes, smok-
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Recent studies have reported a high incidence of diabetes in an indigenous Australian population, accompanied by a significant increase in the prevalence of periodontitis (147). Cutler et al. (29) also provided data indicating a link of altered serum lipids in type 2 diabetes with poor clinical measures of periodontitis. Accumulating evidence has also suggested the potential for untreated periodontitis to have a negative impact on the medical management of type 2 diabetes, although these studies are not uniformly consistent in this interpretation (10, 23, 25, 79, 80, 225, 226). Thus, the potential interactions enhancing the morbidity of these two diseases have encouraged an increased interest in broadening the clinical and biologic basis for these relationships (Fig. 4).

Fig. 3. Schematic of relationships among plaque bacteria and host responses linking the periodontium with systemic responses. Several members of the periodontopathic microbiota (A) (e.g. Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Campylobacter rectus) can break down epithelial barriers and invade selected host cells and tissues (B). Bacterial invasion activates local resident cells (C), increases pro-inflammatory mediators and cytokines, and enhances local immune cellular components. The process stimulates systemic inflammatory mediators and immune responses to the microorganisms (D), bacteria translocate to the circulation (E), and immune cell traffic from the site increases (F). The chronic bacterial colonization of the supra- and subgingival environment with its juxtaposed gingival tissue results in both a localized inflammation and an increase in inflammatory mediators and bacteria/components, which have access to the systemic circulation. The localized inflammation and/or infection are manifest systemically within the affected host (G) by changes in the endothelium and vascular responses, increasing atherosclerotic risk.

Local/systemic bacteria (e.g. antigen) & immune/inflammatory relationships at periodontitis sites

Reports of individual patients and small cohorts have suggested that patients with more severe periodontal disease may reflect systemic changes associated with stress (200) and symptoms potentially attributable to more severe bacterial infections (180). A number of studies within the past decade have correlated periodontitis with risk of coronary artery and cerebral vascular disease (9, 37, 75, 134, 150, 217, 240). Of the numerous periodontal variables, which were analyzed in this retrospective analysis, periodontal destruction (periodontitis) appeared to be the most consistent variable associated with coronary heart disease and thus, appeared to be associated with excessive risk of cardiovascular disease or stroke. Moreover, periodontitis patients presented with increased levels of serum fibrinogen (i.e. an acute-phase protein) and elevated white blood cell counts, significant risk factors for coronary heart disease (119). Specifically, the host responses to peri-
odontal disease and cardiovascular diseases was reflected by an increase in the acute-phase proteins (i.e. serum amyloid A and C-reactive protein). Plausible mechanisms for this relationship revolve around the ability of chronic inflammation (e.g. periodontitis) to initiate and perpetuate systemic elevations in various cytokines related to the acute-phase response. Mattila et al. (149, 151), in early studies, showed that dental health was worse in the subjects with myocardial infarction, even after adjustment for age and other major risk factors for coronary heart disease, and found that dental infection was associated with severity of coronary athero-
sclerosis in men after adjusting for major conventional risk factors, including smoking. Five cohort studies have found odds ratios ranging from 1.2 to 2.7 for dental health and new coronary heart disease or coronary heart disease mortality, with most of the results between 1.2 and 1.5 (9, 37, 90, 104). All these studies adjusted for major risk factors, including smoking. In a cross-sectional study of U.S. veterans the measure of dental health most strongly associated with coronary heart disease was periodontitis (131). Thus, there is considerable evidence for a statistical association of poor dental health, particularly periodontitis, with increased probability of develop-

**Fig. 4. Schematic link between periodontitis and diabetes.** The human population demonstrating type 1 diabetes (generally younger individuals) and type 2 diabetes (generally adults, although an increasing number of juveniles and adolescents) demonstrate alterations in insulin production and/or insulin binding having an impact on the body's ability to regulate glucose utilization. Data demonstrate that patients with type 1 and type 2 diabetes have increased frequency and severity of periodontitis, potentially via altered subgingival biofilm components, altered inflammatory responses, and altered wound-healing capacity. However, accumulating evidence supports the possibility that the chronic infection and inflammation of periodontitis may contribute to a decreased ability to manage diabetes and its systemic sequelae.
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...ing coronary heart disease, and the association is independent of other established major risk factors for coronary heart disease (111).

Existing evidence strongly supports the ability of diet to modify serum lipid/lipoprotein profiles, accelerate atherogenesis, and increase the associated risk of coronary heart disease. The strength and consistency of the epidemiologic association of periodontitis with coronary heart disease similarly suggest that the two diseases are not independent events and are linked by yet undetermined physiological mechanisms. The association may occur because there is a common cause for the two diseases as illustrated in Fig. 5. If this is the true relationship, the prevention or treatment of periodontitis would have no effect on atherosclerosis. On the other hand, if the relationship follows the pattern represented in Fig. 6, periodontitis would accelerate or augment atherosclerosis and thereby increase the risk of coronary heart disease. In this situation, prevention or treatment of periodontitis would be expected to retard the progression of atherosclerosis and reduce the risk of coronary heart disease. The currently available evidence from cross-sectional, case-control, and prospective epidemiologic studies does not permit us to choose between the two types of associations illustrated in the figures. Resolution of this question is important because coronary heart disease remains an important health problem for American adults; and evidence showing that periodontitis contributes to risk of coronary heart disease would provide a powerful impetus for programs designed to prevent or treat periodontitis.

More recent studies have also supported the concept that circulating nonself materials (e.g. oral bacteria and/or their products) may not only contribute to inflammation and wound healing in the periodontium via a similar mechanism, causing increased risk for myocardial infarction (e.g. altered vascular responses, atherosclerosis, etc.). Therefore, interventive therapy for periodontitis would have little impact on cardiovascular disease events.

**Periodontitis & Cardiovascular Disease**

(2 diseases/similar underlying risks)

![Diagram of the association between periodontitis and cardiovascular disease](image)

Fig. 5. Schematic association of periodontitis and cardiovascular diseases. Individuals may be predisposed to both of the disease entities because of common underlying risk factors (e.g. atherogenic diet, genetic control of serum lipids, smoking, etc.). These dietary and/or genetic components may alter the microbial challenge and inflammatory response/wound healing in the periodontium via a similar mechanism, causing increased risk for myocardial infarction (e.g. altered vascular responses, atherosclerosis, etc.). Therefore, interventive therapy for periodontitis would have little impact on cardiovascular disease events.
to cardiovascular disease, but also affect fetal development, if they are not successfully removed by the reticuloendothelial system. The detection of acute-phase reactants in the serum of periodontitis patients suggests that noxious materials from the oral cavity may have the capacity to challenge various tissue and organ systems, in addition to the liver. Recent studies have provided support for an association between periodontal infections and preterm birth/intrauterine growth retardation (Fig. 7). Of particular interest, various oral microorganisms have been associated with bacterial vaginosis, such as *Prevotella* spp., *Porphyromonas* spp. and *Bacteroides* spp. Likewise intra-amniotic infections, including those by *Fusobacterium* spp., viridans streptococci, and at least 14 cases of chorioamnionitis associated with *Capnocytophaga* spp. infection, have been identified, associated with preterm labor (9, 39, 90, 92, 93, 104, 111, 119, 131, 134, 142, 149, 151, 168, 169, 240). A case–control study involving women in North Carolina demonstrated that the preterm birth/intrauterine growth retardation group had significantly more periodontal disease than the control group. Multivariate logistic regression models were used to control for other risk factors and covariates; periodontitis was a statistically significant risk factor for preterm birth/intrauterine growth retardation with adjusted odds ratios of 7–8 (165). Both Davenport et al. (34) and Dasanayaka (33) reported more severe periodontitis in preterm birth/intrauterine growth retardation mothers than controls in different ethnic populations. Damare et al. (30) showed a positive correlation between amniotic levels of prostaglandin E2 and IL-1β and their concentration in the GCF Re-

Periodontitis & Cardiovascular Disease (PD provides etiologic risk factors)

Fig. 6. Schematic link between periodontitis and cardiovascular diseases. Individuals at risk of periodontitis (e.g., subgingival pathogenic biofilm, genetic inflammatory hyperresponsiveness), induce a systemic challenge via bacterial translocation and/or inflammatory mediators that alter vascular responses. These risk factors combine with, such things as an atherogenic diet and/or genetically determined serum lipid profiles to enhance the expression of atherosclerosis and cardiovascular disease events. In this model, interventive therapy for periodontitis would be predicted to have a positive impact on the frequency/severity of cardiovascular disease risk and events.
Recent studies have provided additional evidence for this linkage. Mitchell-Lewis et al. (153) identified a linkage of preterm/low birth weight in young minority women, that appeared to be associated with gingival inflammation. Jeffcoat et al. (100) reported on a study of over 1000 pregnant women and demonstrated an increased odds ratio for preterm birth and/or low birth weight with increasing severity of periodontitis. In addition, Lopez et al. (133) provided data on a randomized treatment study of periodontitis in pregnant women. A significant decrease in preterm birth and/or low birth weight infants was demonstrated in the treated group.

Common mechanisms (Fig. 8) could be proposed that would link the localized periodontal infection and the accompanying inflammatory and immune response with diabetes control, accelerating the progression of atherosclerosis, and initiating a preterm birth event. Three major methods could link these various systemic sequelae.

- Translocate bacteria to atherosclerotic lesions or chorioamniotic tissues;
- Secrete pro-inflammatory cytokines (IL-1β, IL-6, tumor necrosis factor-α) in circulation, which may stimulate acute-phase responses, injure arterial endothelium, activate chorioamniotic tissues responses, or alter pancreatic cell functions;
- Augment existing risk factors and intervening variables, particularly oxidation of low-density lipoprotein by increased oxidant load or depletion of antioxidants, levels of biomolecules initiating the molecular aspects of the birthing sequence, or change systemic glucose utilization via stress to the host system.

**Fig. 7. Schematic link between periodontitis and preterm birth/intrauterine growth retardation (PTB/IUGR).** Expectant mothers with periodontal infections could seed bacteria and/or inflammatory mediators into the systemic circulation. These stimuli would trigger alterations in the amniotic tissues or the fetus to enhance the incidence of negative birthing outcomes. TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; PGE₂, prostaglandin E₂.

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Fig. 8. Summary of potential relationship between periodontitis and systemic diseases. Both genetically determined risk and sociobehavioral risk factors have been suggested as underlying threats for the development of diabetes (types 1 and 2; T1DM and T2DM), cardiovascular disease, coronary heart disease (CHD), preterm birth/ intrauterine growth retardation (PTB/IUGR), and periodontitis (left hand side of figure). Thus, the associative relationships would be through exposure variables, such as, infection and dysregulated inflammation. This contrasts with existing genetic and sociobehavioral risks, combining with risks related to periodontitis (e.g. infection, inflammation, wound healing) synergizing to increase the incidence and severity of systemic diseases (right hand side of figure).

Conclusions

The predominant polymicrobial infection of mankind is expressed clinically as periodontal disease, which afflicts almost half of the population by 50 years of age, and is related to development of a microbial biofilm colonizing the subgingival sulcus (187). The suggested mechanisms of pathogenesis of periodontal diseases are varied, in most part the result of the complex microbial community consisting of numerous bacterial taxa, ranging from aerobes through fastidious anaerobes, fungi, and even viruses at disease sites. This established biofilm initiates a host response in the local tissues comprised of acute and chronic inflammatory mechanisms. The humoral immune response is undoubtedly a component of this response, and appears to be a principal player associated with destructive disease sites. The antibodies of the humoral immune response detected in the GCF bathing the tooth are clearly comprised of locally synthesized molecules. These antibodies are generally specific for bacterial components and reflect the local colonization/infection by particular species. Variation in GCF antibodies across subgingival sites in the oral cavity reflects the biological variability in clinical presentation and existing microbial ecologies. The level of antibody in the GCF appears to provide some immunoregulation for the local infection and qualities of the antibody reflect the level of infection and potentially the progression of the disease. With recent data supporting a contribution of oral infections to general systemic health, the ability to utilize the GCF antibody response to control infections at the local oral sites would appear to have merit. However, the most effective means for regulating this local response, the optimal characteristics of this response, and broad strategies for using GCF antibody to maintain local and systemic homeostasis remain elusive, and in need of well-designed clinical investigations.

References


39. Douvier S, Neuwirth C, Filippuzzi L, Kisterman JP. Chorioamnionitis with intact membranes caused by *Copenhagenia sp*.


71. Geivelis M, Turner DW, Pederson ED, Lamberts BL. Measurements of interleukin-6 in gingival crevicular fluid from...


141. Mackler BF, Waldrop TC, Schur P, Robertson PB, Levy BM. IgG subclasses in human periodontal disease. I. Distri-

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174. Oleksy A, Banbula A, Bugno M, Travis J, Potempa J. Proteolysis of interleukin-6 receptor (IL-6R) by Porphyromon-


