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Hypothesis: The Humoral Immune Response to Oral Bacteria Provides a Stimulus for the Development of Rheumatoid Arthritis

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Abstract—Rheumatoid arthritis (RA) and adult periodontitis share common pathogenetic mechanisms and immunologic and pathological findings. One oral pathogen strongly implicated in the pathogenesis of periodontal disease, *Porphyromonas gingivalis*, possesses a unique microbial enzyme, peptidylarginine deiminase (PAD), the human equivalent of which has been identified as a susceptibility factor for RA. We suggest that individuals predisposed to periodontal infection are exposed to antigens generated by PAD, with deiminated fibrin as a likely candidate, which become systemic immunogens and lead to intraarticular inflammation. PAD engendered antigens lead to production of rheumatoid factor-containing immune complexes and provoke local inflammation, both in gingiva and synovium via Fc and C5a receptors.

KEY WORDS: rheumatoid arthritis; periodontitis; *Porphyromonas gingivalis*; peptidylarginine deiminase; citrulline.

Adult periodontitis (AP) has recently been linked to a variety of systemic medical disorders, including coronary artery disease, stroke, osteoporosis, and low birth weight. (1–2) However, these associations are based almost entirely on epidemiologic data. Although some shared and potentially pathogenic associations, such as levels of C-reactive protein, have been identified, in most cases there is no obvious common disease mechanism (3). On the other hand, adult periodontitis and rheumatoid arthritis (RA) are remarkably parallel disease processes that share not only some clinical features, but pathophysiologic, epidemiological, and therapeutic features as well.

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Rheumatoid Arthritis and Periodontitis: Common Pathophysiologic Mechanisms

In 1982, Snyderman and McCarty first commented on inflammatory mechanisms common both to rheumatoid arthritis and adult periodontitis. Both RA and AP are characterized by self-sustaining inflammation in a fluid-filled compartment adjacent to bone, in which inflammatory cells and other phlogistic factors lead to common clinical manifestations (pain, swelling, tenderness) and, eventually, to erosion of the adjacent bone (4).

Patients with active RA demonstrate significantly increased frequency and severity of periodontal disease, particularly more gingival bleeding and calculus formation, as well as more tooth loss and decreased alveolar bone height as compared to unaffected controls (5–6). In a recent pilot study, it was reported that subjects with AP had a fourfold increased incidence of RA, although the study was seriously flawed by the use of a self-reported diagnosis of RA (7). In a subsequent study, this same group performed careful dental exams on 65 subjects with validated RA and matched controls. They found that the RA patients had more missing teeth and deeper gingival

pockets when compared to the controls (8). A comprehensive comparison of RA and AP has also been published which concluded that "a general and underlying dysregulation of the host inflammatory response is present in both conditions seems very likely" (9). It has been suggested that anti-inflammatory treatment given for RA interferes with periodontal disease and may have minimized the correlation between the indices of destruction in RA and periodontal disease (6). The clinical occurrence of periodontal disease in patients with RA has also been attributed to inadequate or abnormal saliva production as a consequence of concomitant Sjogren's syndrome, seen in up to 15% of such patients, possibly through effects of bacterial colonization (10). Smoking, a known determinant of the severity of AP, is also a risk factor for the severity of RA (11). Indirect periodontal effects have been attributed to rheumatoid involvement of the TMJ (12).

In both periodontal lesions and rheumatoid synovium, local immune responses are amplified with recruitment of inflammatory cells from the systemic circulation into the target tissue (gingival mucosa or synovial membrane). Both cellular and humoral immune reactions have been shown to contribute to the pathogenesis of the two diseases. Activation of monocytes by stimulated T-lymphocytes is a major initiator of the production of large amounts of tumor necrosis factor (TNF)-α and interleukin (IL)-1β (13–15). These cytokines further stimulate the expression of adhesion molecules and other inflammatory mediators that amplify the local inflammatory reaction, leading to the generation of proinflammatory mediators, including cytokines, eicosanoids, including induction of COX-2 (which has been implicated in a rat model of periodontitis), proteolytic enzymes, in particular the production of matrix metalloproteinases, activated oxygen, and nitrogen species (13–18). Immune complexes and complement are the final common mediators of inflammation in both conditions (15, 19), with the ultimate result being absorption of adjacent bone (15, 20–21).

Ethnic groups with an increased prevalence of RA such as the Pima Indians have also been identified as having an increased prevalence of periodontitis (22), a finding that correlates with the high prevalence of rheumatoid factor (RF) in this population (23) (see below). Immunogenetic studies of patients with RA have established an association with specific HLA antigens, in particular at the HLA DR4 locus. The strongest association is with a conserved amino-acid sequence corresponding with positions 70–74 of the third hypervariable region of the HLA-DRB1 gene (termed the "shared epitope"), primarily subtypes 0401, 0404, and 0408 in the white population and 0405 in Asians (24–25). This same genetic locus (HLA DR4) has been associated with development of severe and rapidly progressive periodontitis, mainly subtypes 0401, 0404, 0405, and 0408 (26).

Finally, many therapeutic measures for RA are also effective in AP, including the use of nonsteroidal anti-inflammatory drugs, polyunsaturated fatty acids, gold salts, inhibitors of matrix metalloproteinases, and cytokine inhibitors (27–28). Tetracycline antibiotics, recently introduced as adjunctive therapy in the treatment of AP, presumably acting through nonantibiotic properties as inhibitors of matrix metalloproteinases, have been shown to have anti-inflammatory and immunomodulatory properties in patients with RA. Although doxycycline has been approved for use in treatment of periodontitis, minocycline appears to have greater antirheumatic effects (29). An inhibitor of IL-1 was shown to be effective in a primate model of AP; such an inhibitor (Kineret®, Amgen) has been on the market for use in RA for several years. Inhibitors of TNF-α, which have revolutionized the treatment of RA, have been shown to suppress experimental models of AP. Indeed, an editorial review by one of the current authors has suggested that AP serve as a model for testing interventions that might be used therapeutically in RA (27).

Rheumatoid Factor: Pathogenic Molecule or Epiphenomenon?

Anti-γ globulins or rheumatoid factors (RFs) were originally described in the 1940s and have been identified in the serum of 70–80% of patients with RA (30–32). Further analysis has revealed these to be immunoglobulins that form antigen–antibody immune complexes with IgG. Although circulating RF has been considered an important marker for RA and is part of the American College of Rheumatology diagnostic criteria for RA, it is not entirely specific for this disease, being found in other rheumatic and autoimmune diseases. Indeed, RF is found in small numbers in the unaffected population, with a frequency ranging from 1.3 to 4.0% in Caucasians to as high as 30% in some Native American tribes (32). Although these complexes have been directly implicated in the pathogenesis of RA since the early work of Hollander *et al*. (30) and Pope *et al*. (31), it has also been argued that they are merely a marker of sustained immunologic activity(33).

RF can be identified in two major varieties. Low affinity RFs are naturally occurring antibodies of IgM class, with specificity for IgG-Fc determinants and cross-reactivity with other autoantigens. They are produced

by CD5+ B cells in normal subjects and their production is T-cell independent (34). These RFs are coded for by selected germline V genes and are frequently expressed in low-grade chronic B cell lymphoproliferative conditions, such as chronic lymphocytic leukemia, Waldenstrom's macroglobulinemia, and lymphoma. They share similar characteristics with RFs produced in response to polyclonal B cell activation by Epstein–Barr virus or lipopolysaccharide (LPS) (35). The multivalency of IgM RF allows excellent agglutination of latex beads, RBCs, and *in vivo* microbial organisms that are coated with microbe-specific IgG antibodies. This leads to large immune complexes, which are poorly soluble and rapidly removed by the macrophage-phagocyte system (35–37). In most cases, the RF response to infection is transient. However, persistent organisms, such as viruses that cause latent or chronic infections, e.g., hepatitis C or the herpesviruses, and bacteria prevalent in the environment, such as the oral flora and enterobacteriaceae, may serve as sources of persistent high titers of RF. Organisms with strictly ordered, not irregularly arranged, antigens and coated with specific IgG dramatically enhance induction of RFs (37). This may explain why particular organisms are more often associated with RF production than others (33, 35, 37). Recent data suggests that RF production can be magnified by simultaneous engagement of B-cell receptors for immunoglobulin and Toll-like receptors (TLRs) for pathogen-associated molecular patterns (PAMPs), which include LPS, microbial lipoproteins, and hypomethylated CpG DNA, commonly found in bacterial DNA, and endogenous ligands released from damaged or stressed host cells. The ability of microbial PAMPs to engage TLR or upregulate TLR expression, potentially creating a synergy with immune complexes, may explain the association of infection and RA flare (38).

Although most RFs are IgM, high affinity RF can be antibodies of any Ig class. They are produced by antigen driven B cells, after undergoing affinity maturation and somatic hypermutation of the V genes of the light and heavy chain immunoglobulin genes (39). Soluble RFs may interact with minute amounts of immune complexes in the synovium or joint fluid enhancing the formation of pathogenic immune complexes. While small dimeric IgG-RF complexes may be too small to invoke a systemic complement response, these complexes enter the joint through fenestrated vessels (40). Some of the complexes remain as dimers, but others further self-aggregate to form larger immune complexes via hydrophobic interactions and become trapped within the synovial tissue, triggering complement stimulation and cytokine release by mononuclear cells present in synovium (32–33). It is these higher affinity RFs, typically IgA RF and self-aggregating 7s IgG RF, that have consistently been shown to be strong predictors of disease severity and radiographic progression and have been linked with specific features of RA (33). Although RF may not be essential for the development of RA, current evidence strongly suggests that RF represents an important pathway for the amplification of joint disease in RA (15). Below, we review the possible role of RF in initiating and/or sustaining the disease process in AP.

Rheumatoid Factor in Periodontal Disease

RF has been detected in the gingiva, subgingival plaque, saliva, and serum of patients with various periodontal diseases, particularly in patients with AP (41–45). However, the characteristics and precise function of RF in periodontal disease has not yet been established. B cells and plasma cells are abundantly present in the cellular gingival infiltrate in all types of periodontitis. Included among the B-cell component are $CD5^+$ B cells, which are known to produce natural autoantibodies, such as IgM-RF (46). The presence of locally produced immune complexes has been substantiated by perivascular and basement membrane immunofluorescent staining (19, 47). Hara *et al*. have hypothesized that the binding of RF to low-affinity IgG and the resultant activation of complement may allow RF to modulate the immune response to bacteria in periodontal lesions (32, 48).

To address whether oral pathogens would induce the production of RF, Hara *et al*. measured serum IgM- and IgG-RF levels by ELISA in BALB/c mice after intraperitoneal injection of purified LPS from various oral bacteria, including *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum*, and *Capnocytophaga ochracea*. The bacterial components studied increased both serum IgM and IgM-RF. Injection of *F. nucleatum* and *C. ochracea* LPS resulted in the greatest increases in both IgM- and IgG-RF levels. The variable ability to induce RF was felt to be due to the differing chemical composition of LPS from the various bacteria, with a larger content of fatty acids and lower percentage of carbohydrate in *A. actinomycetemcomitans*, *F. nucleatum*, and *C. ochracea* (48). This corroborates the results of Thé who reported a correlation between the presence of serum IgM-RF and serum antibody titers to *F. nucleatum* and *C. ochracea*, demonstrating that patients with periodontitis and serum RF had significantly higher serum IgM and IgG antibody levels against antigens from sonicated

extracts of *Capnocytophaga* species and *F. nucleatum* as compared to those who were seronegative (45).

In several experiments analyzing RF from patients with periodontal disease and patients with RA, it was found that IgM-RF reacted with various oral microorganisms. In the RA population, four of five samples reacted with *C. gingivalis* and three with *F. nucleatum* (even though none had elevated serum antibody to *F. nucleatum*), whereas in the periodontitis group, seven of 14 reacted with *C. gingivalis* and 11 of the 14 with *F. nucleatum*. After allowing IgG antibodies to bind to oral microorganisms *in vitro*, a marked increase in the amount of IgM-RF binding IgG was observed as compared to IgG bound nonspecifically (49). This augmentation of binding would be anticipated since the determinants on IgG to which RF is attracted are presumably more accessible after binding to bacterial antigens. To examine the specificity of the IgM-RF preparations for human IgG molecules with different antibody specificities, IgG samples with specific activity against the various oral microorganisms were prepared. However, no increased binding of IgM-RF was observed in relation to any specific antibodies, suggesting that the RF activity was not directed against antigen-specific determinants on the IgG molecules (49).

Because earlier experiments had suggested shared antigenicity between human IgG and oral microbes, additional studies examined the response of rabbit IgG directed against periodontal pathogens and found that these rabbit IgGs preferentially associated with human IgG. These data suggest that the increases in IgM-RF noted in patients with periodontitis reflect chronic antigenic stimulation by specific oral microbes with cross-reactive epitopes, which then facilitate the clearance of IgG-coated bacterial pathogens (49). The sum total of these experiments is that AP is a close mimic of RA with respect to the involvement of immune complexes and complement acting via their discrete receptors (15).

Citrullinated Proteins Link Rheumatoid and Periodontal Inflammation

Recently, other antigens resulting in autoimmune complex formation with greater specificity for RA have been identified.

Antibodies directed against structures produced by keratinized epithelia have been identified in sera of some patients with RA. This family of antibodies, termed the anticyclic citrullinated peptide (CCP) autoantibody system, includes antiperinuclear factor, antikeratin antibody, antivimentin (formerly known as Sa antigen), and antifilaggrin antibody. These antibodies were originally described in 1964 (50) as IgG binding to perinuclear keratohyalin granules in the cytoplasm of human buccal mucosal cells and again in 1979 (51) binding to a similar antigen in the cornified layer of rat esophagus epithelium. This antigen has also been identified in the cornified layer of human epidermis and is felt to represent either filaggrin (filament aggregating protein), formed during late differentiation of mammalian epithelial cells and involved in regulation of cytokeratin intermediate filament aggregation (52), or its precursor, profilaggrin. These proteins become antigenic through conversion of arginine to citrulline, which appears to be an essential component of the epitope that their respective autoantibodies recognize. Citrulline, a nonessential amino acid, is not incorporated into proteins during translation, since no citrulline tRNA exists. The presence of citrulline in proteins, therefore, always represents the result of posttranslational modification. Citrullinated residues are generated by deimination of the guanidino group of carboxyterminal arginine residues on a variety of peptides, producing citrulline and free ammonia, by an enzyme known as peptidyl arginine deiminase (PAD; EC 3.5.3.15) (53). It is believed that there are at least four strongly related isoforms of PAD in mammals, each with a different tissue distribution (54).

Antifilaggrin antibodies are the most disease-specific autoantibodies identified in patients with RA. Both their presence and titer correlate with disease activity and severity. However, since filaggrin is identified exclusively in cells of epidermal origin and antifilaggrin antibodies, which have been identified in rheumatoid synovium and are felt to be synthesized locally by plasma cells resident within rheumatoid pannus, react to antigens identified within the joint, the specificity for these antibodies is likely to be to other cross-reacting antigens (53–54). The local presence of anti-CCP producing cells in rheumatoid joints suggests the local antigen-driven maturation of antigen-specific B cells. Monocytes, recruited to inflamed synovium by chemoattractants, may be responsible for PAD production after differentiation into tissue macrophages, supporting the local production of citrullinated antigens (55). Although citrullination is predominantly observed in proteins of the cytoskeleton, it also seems to represent a general regulatory mechanism, which particularly occurs during apoptosis. The absence of deiminated intracellular protein in synovium from other inflammatory and noninflammatory joint diseases highlights its specificity for RA (53–54).

Deimination converts positively charged arginine residues to polar but uncharged citrullines. These sites become potential targets for IgG antibodies (53). Recent studies have suggested that the target epitopes in synovium are citrullinated vimentin or variants of the α and β chains of fibrin that have been subjected to posttranslational deimination (50, 53, 56–57). Deposits of fibrin and fibrin-related extracellular and intracellular deposits have long been recognized in inflamed rheumatoid synovium (58). Indeed, over 40 years ago, Dumonde and Glynn induced chronic arthritis in rabbits by intraarticular injection of either heterologous or autologous fibrin into previously sensitized animals (59). The model has been more recently revived. Sanchez–Pernaute proposed a disease-specific role for fibrin as an autoantigen, with "aberrant reactivity of synovial fibroblasts to adherent fibrin clots" as the inciting insult (60). The prolonged and complicated degradation of fibrin in the joint cavity, which includes citrullination, as opposed to plasmin-induced fibrinolysis, results in the exposure of new epitopes to immunocompetent cells within the synovium (53).

Although recognized as quite specific for RA, antiperinuclear factor and antikeratin antibody assays (typically measured by indirect immunofluorescence (IIF)), never gained wide acceptance due to inconsistencies in test results. The current assay techniques, utilizing enzyme or line immunoassays, involve use of a synthetic citrullinated peptide as the target antigen. The sensitivity of the assays has been increased to 80% by using cyclic citrullinated peptide. The anti-CCP antibodies are typically IgG and have a specificity of 98% for RA. The presence of these antibodies has been shown to have high predictive value for RA several years prior to disease onset (61) and have been associated with more severe clinical outcomes. Additionally, greater radiologic damage is likely to develop in patients who are positive for anti-CCP than in patients who are negative (61). Interestingly, the combination of the presence of RF and anti-CCP, has been shown to be highly predictive of severe, progressive disease (62).

The link between anti-CCP and RA becomes even more striking in light of recent studies that demonstrate that citrulline present within the HLA binding peptide enhances the peptide-MHC affinity and leads to activation of CD4+ T cells in HLA DRB1 0401 transgenic mice. This suggests that the citrullination status of a particular protein may modulate the immune response against that antigen (63–64).

The association of anti-CCP with RA becomes stronger mechanistically in light of recent genetic linkage studies from Japan (65). A genetic susceptibility locus for RA, previously identified on chromosome 1, has been shown to include four PADI genes. Results of a case-controlled linkage disequilibrium study revealed that the gene encoding one of the forms of PAD4 is associated with increased stability of transcripts and is associated with levels of anti-CCP conferring susceptibility to RA (65).

Porphyromonas gingivalis: Premier Pathogen in Periodontal Disease

The presence of *P gingivalis*, a gram-negative, nonmotile, facultative anaerobe, is strongly correlated with the prevalence of adult-onset periodontitis. In nonhuman primate models of periodontitis, the implantation of *P gingivalis* in the oral cavity has been shown to be sufficient for the development of the disease (66). The organism produces a number of virulence factors, such as cysteine proteases, hemagglutinins, LPS, and fimbriae, which enable the bacterium to colonize and invade periodontal pockets (67). The growth and survival of *P gingivalis* is supported by the degradation of periodontal tissues by either host or bacterial proteinases. An essential family of bacterial proteinases are the arginine-specific cysteine proteinases, gingipains-R, which are responsible for the activation of prekallikrein to initiate production of bradykinin (68); they can also activate factor X, thus generating thrombin (69), and inactivate the cytokines TNF-α, IL-1, and IL-6 (70), resulting in increased vascular permeability and crevicular fluid flow, helping to sustain the organism (71).

Arginine deimination is critical due to the reliance of these organisms on ammonia generation to tolerate and neutralize acidic environments, optimize the activity of natural proteinases, inactivate biologically relevant peptides such as hemagglutinins, and promote production of ATP by other enzymes in this pathway, all associated with their pathogenicity (68, 72) Furthermore, ammonia, through the nitric oxide synthetase (NOS) pathway, may generate nitric oxide (NO). NO is a reactive nitrogen species with pleiotropic properties that has been shown to function in signaling pathways and has been implicated in the pathogenesis of periodontal disease, in addition to contributing to cartilage degradation in joint diseases (16). NOS activity is increased in localized aggressive periodontitis and is negatively correlated to chemotaxis response (73).

P gingivalis is the only prokaryotic that produces PAD (68). However, microbial PAD is not evolutionarily related to the vertebrate family of PAD enzymes (74).

Microbial PAD can convert both peptidylarginine and free L-arginine, and shares sequence homology with several other arginine deiminases (e.g., gingipains, mentioned above). Thus, PAD activity by *P gingivalis* may promote the growth of the pathogen in the periodontal pocket, initially by enhancing its survivability and then by assisting the organism in its circumvention of host humoral defenses (68). Degradation of fibrin, mediated at least in part by microbial PAD, provides a nutritive milieu of peptides to sustain the growth of the oral pathogens that induce periodontal injury (71). The capacity to break down fibrin via PAD may in fact promote survival of *P. gingivalis*, since fibrin-related extracellular deposits constitute a major component of the fibrous tissue band surrounding the lesion (75). A negative correlation has been established between the proportion of intact fibrin in crevicular fluid and the clinical measures of periodontal inflammation (76). Concentrations of fibrin degradation products have been measured at levels comparable to those seen in rheumatoid synovial fluid (76).

CONCLUSIONS

One common explanation for the development of autoimmune disease is that infectious agents provoke immune responses to altered self-antigens in genetically predisposed individuals (77). The association between antecedent infections and rheumatoid arthritis has long been suspected but never proved: Lyme arthritis without the spirochete as it were (78). But, absent a temporal clustering of the clinical onset of disease, the infectious agent of RA—if it exists—must be one not commonly recognized as a pathogen, or undetectable by routine methods, or an organism not usually found in the joint. Since repeated attempts have failed to find a unique organism in RA synovial tissue, does the septic stimulus for RA dwell in the periodontal lesion?

We have summarized several pathogenetic mechanisms common to both RA and AP:

1. The same immunogenetic markers are associated with both disease processes (HLA-DR4).
2. Oral pathogens promote production of rheumatoid factor, both directly (antibacterial) and indirectly (ligation of TLR), both locally (in gingival tissue) and systemically (in serum).
3. Local immunologic events ultimately lead to generation of immune complexes and complement acting via Fc and complement receptors, activate phagocytes in the chronic lesions of both RA and AP.
4. One oral pathogen (*P. gingivalis*) possesses a unique microbial enzyme (PAD), which has the capability of deiminating arginine in fibrin in the periodontal lesion, a characteristic shared with human PAD, an established susceptibility factor for RA.

We suggest that individuals predisposed to periodontal infection are exposed to citrullinated antigens that, in the proinflammatory context of periodontitis, become systemic immunogens. Individuals with PADI4 are capable of deiminating intraarticular proteins, including fibrin. Epitope spreading allows for the generation of antibodies to structurally related, but not necessarily identical, peptides. Indeed, PAD engendered antigens elicit IgG antibodies that may bind in a strictly ordered array, allowing them to become the antigens for complement-fixing IgM, anti-IgGs, or RF immune complexes. Together with phagocytic cells such as neutrophils and macrophages, immune complexes are critical to the pathogenesis of RA and AP; their effects are mediated by a complex cascade involving complement activation and stimulation of phagocytes via C5a and Fc receptors. These mechanisms result in a release of mediators of inflammation and joint destruction: cytokines, metalloproteinases, eicosanoids, and reactive oxygen and nitrogen species, ultimately leading to the destruction of adjacent bone.

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