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Aggressive Periodontitis: microbes and host response, who to blame?

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A paradigm shift several decades ago elucidated that aggressive periodontitis (AgP) was not a degenerative disorder but a rapid progressive form of plaque-induced inflammatory periodontal disease. Ensuing years of research have led to linkage analysis identification of specific genetic defects responsible for AgP in some families and to the finding that subgingival detection of *A. actinomycetemcomitans* JP2 clone is a predictive factor for disease onset and progression. However, rather disappointingly, these 'proven' risk factors are only detected in a small subset of AgP cases. Recent advances are leading to a new paradigm shift, with the realization that genetically-driven dysbiotic changes in the subgingival microbiota may predispose to a cascade of events leading to the rapid periodontal tissue destruction seen in AgP. This review tries to dissect the existing literature on the host response-microbial axis of AgP and to propose possible pathogenic pathways in line with current theories.

Introduction

In 1999, the American Academy of Periodontology (AAP) introduced the controversial term "Aggressive Periodontitis" (AgP) to define a group of destructive periodontal diseases with a rapid progression.[1] This definition aimed to encompass previous definitions of early-onset periodontitis, juvenile periodontitis and rapidly progressive periodontitis, using a terminology ("aggressive") rather unusual in the medical field. This put the emphasis on rapidity of progression (rather than necessarily on age of onset) and perhaps on the difficulty of treating it. Along with rapid progression, systemic health and familial aggregation were considered the main features, following concepts already described three decades earlier.[2] Among other secondary features, a microbiological element was introduced, in theory to help in the differential diagnosis with the more common chronic periodontitis (CP). The authors of the consensus statement reported that "elevated proportions of *Actinobacillus actinomycetemcomitans* (now *Aggregatibacter actinomycetemcomitans*) and in certain populations of *Porphyromonas gingivalis*" were typical of AgP.[1] This reflects the enormous efforts of previous decades to identify a microbiological culprit able to cause rapidly progressive periodontitis in young and healthy individuals. Identification of a specific disease-initiating microbe would help in the diagnosis and treatment of AgP, targeted at the elimination of the responsible pathogen. On the other hand, it was also thought that deficiencies in the host response could be at the basis of the rapid periodontal destruction. Aggressive periodontitis was further divided into a localized form (LAgP) typically affecting first molars and incisors, and a more generalized form (GAgP), not necessarily deriving from the former,[1] but possibly expression of a different pathogenic process. Although affecting a relatively small proportion of the population compared with CP, the rapidity of progression and high risk of tooth loss at a young age make AgP a very unique and relevant disease.[3] The next sections will review the evidence to accept or reject the notion that AgP has a distinct microbiological pathogenesis from CP and to help decide whether microbiological or host response factors are more to blame for its onset and progression.

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AgP- Microbiological Studies

The first term used to define cases of early-onset destructive periodontal diseases was "periodontosis."[4] This should perhaps not surprise us, since no clear "inflammation" element can be macroscopically distinguished in many of these cases, with disproportionate periodontal destruction compared with the amount of local deposits, especially in LAgP cases.[5] Hence, the emphasis was on a possible degenerative disease leading to bone resorption and tooth mobility. While the 1960s brought the evidence to confirm that dental plaque is the etiological factor of periodontal diseases,[6,7] crucial studies in the 1970s investigated a possible microbiological cause of what we now call AgP. As "non-specific"[8] and then "specific"[9] plaque hypotheses for periodontitis developed, it was now becoming clear that AgP was also a plaque-induced gingival disease. Listgarten, by light and electron microscope analysis of teeth extracted from "periodontosis" cases, identified "small clumps of Gram-negative coccoid bacteria and neutrophils in varying stages of disintegration." He also observed fewer morphologic forms compared with CP.[10] Newman and co-workers detected

Gram- anaerobic bacteria including *Bacteroides, Campilobacter, Selenomonas, Fusobacterium* with an additional 45% not identified.[11] A milestone in the microbial studies on AgP came with the discovery of the role of *A. actinomycetemcomitans*, found to have pathogenic abilities and characteristics which allowed its inclusion as a periodontal pathogenic bacterium.[12] In a large study in the United States, *A. actinomycetemcomitans* was detected in nearly all the analyzed LAgP patients, and in contrast in fewer healthy (16.9%) or chronic periodontitis subjects (20.8%) as assessed by culture and immunofluorescence serotyping.[13] A more established role for *A. actinomycetemcomitans* became clearer with the evidence of a correlation of its elimination with treatment response [14] and the finding of an elevated serum antibody response to this bacterium in patients with LAgP.[15,16] The next milestone was represented by the discovery, in a young case of LAgP, of the *A. actinomycetemcomitans* JP2 clone,[17] characterized by a 530-base-pair deletion in the leukotoxin operon, associated with increased leukotoxic activity.[18] A different pathogenic potential was also identified across the various *A. actinomycetemcomitans* serotypes.[19] *A. actinomycetemcomitans* has shown clear association with onset of AgP in a 1-year prospective study in the United States, where patients with detectable *A. actinomycetemcomitans* at baseline had a higher risk of developing AgP compared with matched subjects who did not harbor it. In particular, 8 out of 38 *A. actinomycetemcomitans*-positive and none of 58 *A. actinomycetemcomitans*-negative subjects exhibited bone loss at the last study follow-up.[20] A similar association was found in a 15-year longitudinal study in Indonesian adolescents and young adults,[21] while no predictive role for *A. actinomycetemcomitans* in disease progression was found in a German study on 105 young male soldiers followed up for 1 year.[22] A prospective study in a Moroccan population of 428 adolescents showed a strong association between the JP2 clone and periodontal attachment loss over a 2-year period.[23] A more recently-published longitudinal study on nearly 400 Ghanaian adolescents showed a similar association between detection of the JP2 clone and attachment loss over 2 years.[24] Interestingly, also detection of highly leukotoxic non-JP2 *A. actinomycetemcomitans* clones was associated with increased diseased progression risk.[25]

However, it also clearly emerged that *A. actinomycetemcomitans* is not universally detected in AgP sites [26] and cases [27] and it is also found in healthy individuals [28] and in healthy sites of diseased individuals.[29] Furthermore, studies have often shown to have a similar prevalence of detection in AgP and CP cases.[30] Hence, *A. actinomycetemcomitans*, but also *P. gingivalis*, are certainly not the only possible pathogens predisposing to AgP. Recent technological advances have led to an improvement in microbiological detection techniques and metagenomic studies in AgP are starting to suggest the possible role of non-cultivable microbes in its pathogenesis.[19] Among putative pathogens, based on increased detection in AgP vs. periodontal health, *Filifactor alocis*,[31] *Centipeda genus*,[32] *Mitsuokella sp.*,[33] *Selenomonas genus*,[32] *Actinobacter baumannii*,[34] *Treponema lecithinolyticum*[35] and even the *Archaea* domain [36] are emerging as associated with AgP. Their potential pathogenic role still needs to be elucidated and scrutinized based on Socransky's criteria,[12] once more literature data become available. Nonetheless, a more variegated picture of AgP-pathogenic bacteria is emerging, which has to be carefully kept in mind in future research. In balance, it seems clear that *A. actinomycetemcomitans* (and particularly the JP2 clone) plays a role in the pathogenesis of AgP, especially LAgP. However, it may be possible that *A. actinomycetemcomitans* plays a strong role in disease initiation and is then replaced by other pathogenic bacteria, presumably obligate anaerobic, as the disease progresses.[37]

The Differential Microbiological Profile of AgP and CP

As we endeavor to understand pathogenic and clinical differences between aggressive and chronic periodontitis, hoping to design more targeted management regimes for these conditions, an interesting insight is given by studies comparing the microbiology of AgP and CP. A systematic review over a decade ago concluded that detection of *A. actinomycetemcomitans* or *P. gingivalis* was not enough to discriminate between AgP and CP. In other words, albeit AgP cases were found to harbor *A. actinomycetemcomitans* with higher frequency than CP cases (62% vs. 28%), detection of these bacteria could not be used as a diagnostic criterion. During the last decade, even more studies have cast doubts on a different microbial profile between AgP and CP. Whether due to limitations in the microbiological techniques used or simply to our inability to clinically distinguish certain cases of AgP from CP, no differences in subgingival microbial detection emerged in several studies.[35,37,38,40] We recently reported results of a study on polymerase chain reaction (PCR) detection of *A. actinomycetemcomitans* or *P. gingivalis* from subgingival plaque samples of 267 periodontitis patients (84 diagnosed with AgP and 183 with CP).[30] In this patient sample, CP cases had higher detection of both bacteria compared with AgP, although the differences were not statistically significant. However, this study did not provide quantitative measures of these bacteria subgingivally. Quantitative data on numbers of subgingival bacteria, different serotypes or clones and uncultivable microbes will certainly shed further light into the differential microbial profile of AgP and CP.

The Host Response in AgP

A substantial body of evidence has accumulated to suggest large inter-individual variations in the response to dental plaque accumulation, leading to different degrees of gingival inflammation.[41] In line with this concept, already from the initial attempts into research on early onset forms of periodontitis, it became apparent that affected individuals suffered from a metabolic imbalance or from inherited host response defects.[2,42] The first line of periodontal defense is provided by the innate immune response, in the form of neutrophils and macrophages, fibroblasts, epithelial and dendritic cells, which are normally continuously engaged in responses to bacterial plaque in a state of preclinical "physiological" inflammation.[43] When this response is not able to control microbial accumulation, complex

inflammatory cascades are activated and an adaptive immune response is called upon by antigen-presenting cells, with a progressive shift from a predominantly T-cell lesion to a B-cell dominated lesion typical of periodontitis.[43] As evidence of presence of neutrophils in gingival lesions and in root surfaces of AgP cases accumulated,[44,45] it emerged that neutrophils of AgP patients could be responsible for disease predisposition due to an array of suspected malfunctions, including increased adhesion, reduced chemotaxis, increased superoxide and nitric oxide production and reduced phagocytosis.[46-49] These neutrophil features would make individuals more susceptible to periodontitis upon subgingival microbial colonization. The 1999 Workshop also suggested among secondary features of AgP 'Hyper-responsive macrophage phenotype, including elevated levels of prostaglandin E2 (PGE2) and interleukin (IL)-1β'.[5] Recently, an excessive local and systemic inflammatory response has been reported in AgP cases, specific to macrophage inflammatory protein (MIP)-1α [50] and to response to bacterial endotoxin in LAgP.[51] A recent study investigated antibody response to periodontopathogenic bacteria in 119 patients (18 LAgP, 37 GAgP, 37 LCP, 27 GCP). Subgingival plaque samples were analyzed by checkerboard for 11 bacterial species, serum immunoglobulin G (IgG) levels against the same bacteria were investigated and 'infection ratios' (antibody level over the average bacterial colonization) were calculated for each patient. Interestingly, no differences in serum IgG levels to *A. actinomycetemcomitans* were detected. IgG levels to several species including *P. gingivalis*, *Treponema denticola* and *Campilobacter rectus* were highest in GAgP patients and significantly different from LCP and generalized CP, but not from LAgP. No differences in 'infection ratios' between GAgP and LAgP were detected, bringing evidence against the 'weak serum antibody responses' in GAgP patients. The authors concluded that serum IgG responses in GAgP are comparable to those in LAgP, but higher than in GCP or LCP for several species.[52] However, it is important to point out that antibody responses are time-related and a positive titer may just indicate previous rather than necessarily present exposure.

Interestingly, even treated AgP cases exhibited an enhanced inflammatory response in an experimental gingivitis model, suggesting a possible constitutive hyper-inflammatory status.[53] These clues lead to the understanding that, if chronically activated by certain microbial triggers, the host response could contribute to the extensive rapid tissue damage to the periodontium seen in these cases. Genetic studies investigating heritable AgP traits have focused on putative host response genotypes, which would cause constitutive neutrophil abnormalities or dysfunctions in other inflammatory/immune response pathways. Enthusiasm gained from linkage analyses and from the observation of single-gene defects leading to early onset forms of periodontitis has been dampened by the reality that AgP encompasses a large range of conditions clinically undistinguishable but possibly with different initiating factors. For example, the cathepsin C defects leading to a non-syndromic form of early onset periodontitis only affect a very small subpopulation of AgP cases.[54] Equally, the chromosome loci suggested by two separate genetic linkage studies [55,56] may indeed predispose to AgP, but probably just in some sporadic cases or families. Promising putative single nucleotide polymorphisms (SNPs) recently identified in genome-wide studies in the GLT6D1 and vitamin C transporter SVCT1 genes may increase the susceptibility to AgP[57,58] in a complex genetic susceptibility profile interacting with environmental factors. Ethnic variations in the susceptibility to AgP and CP also need to be considered, as they have a strong impact on observed genetic associations (which change across different ethnic groups). Among other genetic variants, pro-inflammatory Interleukin (*IL*)-6 single nucleotide polymorphisms (SNP) [59] may act as risk modifiers by increasing the inflammatory potential upon microbial insults.[60] A moderate association between IL-6 genetic variants and increased detection of *A. actinomycetemcomitans* has been repeatedly shown in different periodontitis populations (both AgP and CP) by this author [12,61,62] and others,[63] underscoring the concept of infectogenomics and genetic dysbiosis.[64] In a study on 12 AgP patients selected based on their *IL6* genotypes, we detected higher *A. actinomycetemcomitans* counts in *IL6* 'haplotype positive' subjects before treatment. Despite a reduction after treatment, these subjects tended to have a sharp increase in counts of *A. actinomycetemcomitans* again at the last study follow-up, 3 months after periodontal surgery, suggesting a strong genetic influence on pocket re-colonization.[65] Further examples of interactions between host genetic and microbial factors in AgP can be found in the *A. actinomycetemcimitans*-leukotoxin activation and secretion of bioactive IL-1β and IL-18 by human macrophages,[66] which might act in synergy with the IL-1β polymorphism to cause an imbalance in the host response. Recently, gene expression analyses of gingival biopsies from 55 AgP cases identified overexpressed genes related to immune responses (esp B cells and Nk cells), apoptosis, and signal transduction when compared with gingival samples from 65 CP patients.[67] This suggests some possible differences in pathogenic pathways between AgP and CP. However, studies investigating differences between AgP and CP identified no evidence of differences in the inflammatory infiltrate [68] or in the cells and processes involved, only suspecting a possibly different shift from T-cell to B-cell lesion in AgP compared with CP.[69]

A compelling example of early onset periodontal destruction is given by localized aggressive periodontitis (LAgP), characterized by a circumpubertal onset and a strong antibody response to periodontal pathogenic bacteria.[5] LAgP affects first molars and incisors, the first teeth to erupt in the mouth at age 6–8. The disease progresses very rapidly, with periodontal attachment and alveolar bone loss but then it often stops even in the absence of therapy,[70] suggesting a possible host response adaptation or the mounting of an effective and 'mature' antibody response to pathogenic bacteria.[71,72] Cementum hypoplasia and a possible role of *Cytomegalovirus* infection during root formation (causing malformation of attachment apparatus) have been hypothesized as a possible cause of LAgP.[73,74] However, evidence for a possible role of viruses in AgP is still controversial.[75] Furthermore, owing to its relative rarity, not many investigations on LAgP have been produced in the literature especially with regards to possible genetic risk factors affecting the host response.

Conclusions

Subjects affected by AgP seem to suffer from a 'primed' host response status particularly susceptible to certain microbial triggers.[53] Within this context, in line with the polymicrobial synergy and dysbiosis hypothesis,[76,77] potential keystone periodontal pathogens could lead to disruption of tissue homeostasis and the subsequent dysbiotic change in the subgingival biofilm may induce AgP. For instance, 'hyper-inflammatory' genotypes (as in IL-1 or IL-6 hyper-producers)[59,78] might predispose to subgingival environments more conducive to the growth of bacteria which grow well in more inflamed area such as *A. actinomycetemcomitans*. This is still a theory, but is substantiated by the association between IL6 genotypes and detection of *A. actinomycetemcomitans* and re-colonization with this bacterium following periodontal treatment.[30,61,62,65] The subgingival presence and pathogenic relevance of specific bacteria and strains such as *A. actinomycetemcomitans* JP2 clone is probably modulated by age but is under a strong host genetic influence, as testified by the prevalence of this clone in people of specific ethnicity, not necessarily dependent on their geographic origin.[79,80] The JP2 has shown strong evidence of association with disease initiation and progression.[20,23] This association seems more evident than previously-reported associations between bacteria such as *P. gingivalis* and *T. denticola* and progression of chronic periodontitis.[81,82] However, the jury is still out on whether JP2 clone may work as a classical exogenous bacterium or if it is involved in a dysbiotic process. Hence, host genetic variants may be responsible for the dysbiotic process or for an altered response to dysbiotic changes and cooperate with keystone bacteria and environmental factors in the onset and progression of AgP. However, it has to be borne in mind that AgP probably covers a range of etiologically diverse but clinically undistinguishable entities originating from a complex web of interactions between microbiological and host response factors.

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