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Review Article

Filifactor Alocis as an Etiotropic Agent in Periodontal Diseases

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Abstract

The review analyzes the modern views on the role of *Filifactor alocis* in the etiology of chronic periodontitis. Investigation of this bacterium, which was discovered in 1985, is complicated by challenges associated with its detection using culture techniques. According to contemporary researchers, there are good reasons for including *F. alocis* in the "red complex" of periodontal pathogens as the most important etiotropic agent of chronic periodontitis. *F. alocis* is a synergist of Porphyromonas gingivalis, the key periodontal pathogen. Due to its involvement in arginine metabolism, pronounced protease activity, and a broad range of virulence factors, *F. alocis* significantly affects the development of a community of periodontal microorganisms (including viruses), thus contributing to the invasion of epithelial tissues by these pathogens. *F. alocis* has many unique properties: it is resistant to oxidative stress in the lesion focus, induces the apoptosis of epithelial cells, degrades the extracellular matrix of periodontal tissues, activates proinflammatory cytokines at the sites where this bacterial species is present, suppresses the defense reactions of neutrophilic granulocytes, and inhibits activation of the complement system. Being uncultivated microorganism *F. alocis* is identified either by methods of metagenomic analysis or by PCR.

Keywords: *Filifactor alocis*; Chronic Periodontitis; Periodontal Pathogens; Periodontal Colonization; Virulence Factors

Introduction

Periodontitis is a polymicrobial disease caused by the exposure of periodontal tissue to the pathogen complex residing in microbial biofilm in the gum pockets, which is accompanied by chronic inflammation and deterioration of periodontal pockets [6,29,38]. It has recently been found that the role of periodontitis it more important than it used to be thought earlier as it was discovered that it has systemic effects and is frequently associated with cardiovascular diseases [21], rheumatoid arthritis [17,61], obesity [51], diabetes mellitus [13], Alzheimer's disease [43], chronic obstructive pulmonary disease [26], atherosclerosis [56], adverse pregnancy outcomes [27], inflammatory bowel disease [32], and colon cancer [19]. Bacteria of the so-called red and orange complexes (Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Porphyromonas endodontalis, Treponema denticola, Prevotella intermedia, and Fusobacterium nucleatum/periodonticum) play the crucial role in etiology of periodontitis [2,5,31,33,60]. It was shown that the combination of these microorganisms directly correlates with the depth of periodontal pockets, and not with the other clinical manifestations of chronic periodontitis [45]. Moreover, many of these bacteria proved the possibility of bacteremia, which in chronic periodontitis reaches 16.7% [4]. Due to the recent advances in metagenomic analysis, the range of pathogens has been significantly broadened and now includes bacterial species that cannot be cultured on growth media, such as *Filifactor alocis*, Dialister pneumosintes, Treponema lecithinolyticum, Solobacterium moorei, Cryptobacterium curtum, Mitsuokella dentalis, etc. [7,29]. *Filifactor alocis* deserves special attention as it

is a periodontal pathogen whose virulent properties indicate that it is a "red complex" bacterium [9,20,24]. It never occurs in healthy people [37]. *F. alocis* ranks third among the most important periodontal pathogens responsible for the development of aggressive periodontitis (45%) and second (after *P. gingivalis*) in terms of its involvement in the development of chronic periodontitis (90%) [9].

The key properties of *Filifactor alocis* and the features of periodontal colonization by these bacteria

F. alocis was isolated from the gingival sulcus of patients with chronic periodontitis in 1985. It was originally attributed to the genus Fusobacterium, but further thorough examination of its properties gave grounds for classifying it as belonging to the genus Filifactor [8]. This bacterium is a Gram-positive rod that is incapable of fermenting saccharides or forming spores and is classified into the group of obligate anaerobes [8,9].

F. alocis is a "difficult-to-culture" microorganism. It grows only in brain heart infusion broth supplemented with yeast extract, L-cysteine, and arginine in the presence of 10% hydrogen (H_2), 10% carbon monoxide (CO_2), and 80% nitrogen (N_2). Hiranmayi *et al.* reported that the growth of F. alocis in the niche of periodontal pocket is stimulated by particular amino acids such as arginine, lysine, and cysteine [29].

As already mentioned, *F. alocis* is not detected in individuals without inflammatory oral cavity diseases [37]. Smokers are the only exception: this bacterium is typically found in the sublingual mucosa microbiome in this cohort [57]. In patients with chronic periodontitis, *F. alocis* bacterium colonizes the apical and middle one-third of the periodontal pocket [9]. This bacterial species is also found in the root canals of teeth having periapical lesions and symptoms of endodontic infection, especially in patients with endodontic treatment failure [23]. Tamura et al. showed that *F. alocis* plays an etiological role as an agent causing peri-implantitis [52].

F. alocis resides within the oral mucosa biofilm and interacts with other microorganisms, such as Streptococcus gordonii, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans, to form microbial communities. Thus, it has been reported that *F. alocis* often colonizes the areas where *F. nucleatum* is concentrated, while *S. gordonii* is an antagonist of *F. alocis* and these bacteria are almost never simultaneously found within the biofilm. Furthermore, *S. gordonii* disrupts the synergism shown by *F. nucleatum* with respect to *F. alocis*. The interplay between A. actinomycetemcomitans and *F. alocis* is strain-specific, so A. actinomycetemcomitans can either stimulate *F. alocis* or not have this effect [59].

However, the strongest synergistic interactions are found between *F. alocis* and the "key" periodontal pathogen, Porphyromonas gingivalis. Coexistence of these bacteria not only mutually potentiates their invasive properties but also significantly facilitates biofilm formation [10]. This is facilitated by the coinciding localization of both pathogens in the marginal part of the periodontal pocket [9,46].

The ability of *F. alocis* to form microbial communities is based on the metabolic features of these bacteria. Thus, *F. alocis* carries the genes responsible for enzymatic involvement of this bacterium in arginine metabolism. The bacterium survives and contributes to the growth of other periodontal pathogens due to this mechanism [10]. Aruni et al. reported that arginine content is appreciably high in periodontal tissues and fully satisfies the nutritional needs of the bacterium upon infection of periodontal pockets [8]. Ammonia is a by-product of arginine metabolism; it protects F. alocis and other periodontal pathogens against the unfavorable acidic environment in the inflamed tissues [18]. As *F. alocis* is involved in arginine metabolism, it additionally requires carbamoyltransferase. Although *F. alocis* does not produce this enzyme, it carbamoyl transferase produced by *P. gingivalis* [8]. Butyrate is an end-product of arginine metabolism and facilitates the recruitment of viruses to the microbial community. Other periodontal pathogens, especially *P. gingivalis*, produce butyric acid that reactivates these viruses [35]. In particular, the feasibility of this mechanism of involvement in the pathological process was demonstrated for the Herpesviridae family [49,50].

It is worth mentioning that *F. alocis* can produce transport proteins that bind to iron ions and convey them inside the bacterial cell [11]. Production of virulence factors in bacteria is an iron-dependent process. The contribution of *F. alocis* to pathogenetic properties of microorganisms causing chronic periodontitis increases incommensurably from this perspective [8]. The metabolic activity of *F. alocis* is associated with its ability to produce hydrogen sulfide, thus contributing to the development of halitosis (bad breath) [14].

Virulence factors of *Filifactor alocis* and their role in pathogenesis of chronic periodontitis

The virulence factors of *F. alocis* are similar to those of other periodontitis pathogens [11]. However, unlike in *P. gingvalis*, they are mostly associated with the protease activity rather than gingipain activity [10]. It should be mentioned that gingipains are the main virulence factors of *P. gingvalis* and exhibit a very broad range of enzymatic activities, including their ability to enhance vascular permeability and have an anticoagulant effect, to cleave the components of the complement system, collagen, and receptors for a number of proinflammatory cytokines, and many other effects [25]. In *F. alocis*, these pathogenetic effects on infected tissues show themselves in a different way. One of the main features of *F. alocis* is that this microorganism belongs to Gram-positive bacteria, with proteolysis playing the key role in biological processes (such as post-translational regulation of gene expression, protein processing, and protein mobility) [9].

The genome of *F. alocis* carries an array of genes encoding 15 different proteases [11]. These proteases protect *F. alocis* against bacteriocins produced by bacterial species inhabiting the normal biofilm microflora [8] and destroy intracellular matrix collagen, thus contributing to the degradation of the periodontal tissues [36] and apoptosis as other periodontal pathogens become able to penetrate through the mucosa [10]. Some proteins of *F. alocis* are involved in fatty acid metabolism [16].

Due to the production of proteases, *F. alocis* is more resistant to oxidative stress in damaged tissues and is more likely to survive in periodontal pockets than *P. gingvalis* [41]. Furthermore, *F. alocis* enhances this resistance in *P. gingvalis* [8]. It is assumed that this resistance enhancement occurs due to the ability of *F. alocis* to disrupt glycoprotein sialylation, which is accompanied by the release of sialic acid that reduces oxidative stress in inflamed tissues [30]. *F. alocis* also releases superoxide reductase, which facilitates the growth of this bacterium in the presence of hydrogen peroxide [8]. One of the unique genome organization of *F. alocis* include its possession of 3-methyladenine DNA glycosylase, that is involved in oxidative and nitrosative stress resistance in other pathogenic bacteria [48].

P. gingvalis significantly facilitates penetration of *F. alocis* into the mucosal epithelial cells via the vesicular mechanism of endocytosis stimulation in bacteria [12]. *F. alocis* also contributes to bacterial invasion of the cells. Hence, *F. alocis* reduces the efficiency of the ubiquitin system of the host cells via aminopeptidase production, thus suppressing the mechanisms of intracellular degradation of protein structures and increasing the chances for intracellular survival of bacteria [44]. In addition, *F. alocis* bacteria facilitate filamentation of microvilli of epithelial cells by binding to the epithelial surface via adhesins, thus ensuring endocytosis of both *F. alocis* and associated bacteria [1]. As it penetrates inside the cell, *F. alocis* can activate oncogenes [18]. Furthermore, it can induce apoptosis of epithelial cells via activation of caspases 3 and 9, thus increasing epithelial permeability for various pathogens [41].

Thus, while the most important pathogens were tested, the panel could have included more species particularly the newer pathogens such as *Filifactor alocis* [2]. There was a positive correlation between samples of subgingival plaque and saliva before and after periodontal treatment with respect to relative abundance of specific periopathogens, among them *Filifactor alocis* [15].

Filifactor alocis and innate immune response

Secretion of proinflammatory cytokines (interleukins-1β and 6, tumor necrosis factor α) supporting chronic inflammation and apoptosis in endothelial cells is an important pathogenetic component of *F. alocis* infection [41].

It was shown using experimental models that local inflammatory response caused by *F. alocis* is accompanied active migration of neutrophilic granulocytes into the lesion area. The neutrophilic response can subsequently become systemic [58]. *F. alocis* was shown to reliably induce directed neutrophil migration into the periodontal tissues in humans via induction of interleukin-8 produced by epithelial cells. This bacterium can interact with Toll-like receptors 2 and induce degranulation of these cells, which may facilitate dysbiotic responses in the periodontium and damage tissues by granule contents of activated neutrophils [6]. It was also found that *F. alocis* can disrupt the formation of neutrophil extracellular traps (NETs), the key defense reaction of these cells preventing pathogen spread [5].

Interaction of periodontal pathogens with cells of innate immunity of monocytic origin (macrophages) is carried out by various mechanisms through different receptor systems. Using human peripheral blood monocytes and murine bone marrow-derived macrophages from wild-type and Toll-like receptor-specific knockouts, Marchesan *et al.* demonstrated that heat-killed Porphyromonas

endodontalis, Porphyromonas gingivalis, and Tannerella forsythia mediate high immunostimulatory activity. Studies using mesothelial cells from NOD1-specific knockouts and NOD2-expressing human embryonic kidney cells demonstrated that *Filifactor alocis* exhibit robust NOD1 stimulatory activity. These studies allowed to provide important evidence on newly-identified putative pathogens in periodontal disease pathogenesis showing that these bacteria exhibit different immunostimulatory activity via TLR4 and NOD1 [39].

Complex systems with the participation of *F. alocis* controlled by response regulators protect against oxidative and nitrosative stress induced by phagocytic cells. The combination of these multifaceted strategies would provide a comprehensive defense and support system against the repetitive host immune response to promote microbial persistence and disease [28].

Although *F. alocis* mostly cause a local inflammatory response, this bacterium has a mechanism ensuring its survival in the presence of blood serum. *F. alocis* produces a unique protein FACIN, which binds to the third component of complement (C3) and suppresses all the complement activation pathways so that the complement can no longer cause bacterial lysis [34].

The features of the adaptive immune response against *F. alocis* have not yet been studied in detail.

Diagnostic aspects of *Filifactor alocis* detection

Currently, the problem of periodontal disease and its relationship with other pathological conditions requires great attention. Given the fact that chronic periodontitis is a polymicrobial disease, special importance is attached to the formation of microbial communities and their influence on the course of the pathological process [40]. The presence of the pathogenic properties of *F. alocis* and its ability to influence the invasive ability of concomitant pathogens makes its detection important not only for the processes of studying its role in the development of periodontal tissue damage but also in the clinical practice of dentists.

Methods of cultivation are the basis of traditional microbiological diagnostics of dental diseases, however, the majority of pathogens, including *F. alocis,* belong to the category of uncultivated and cannot be detected by this method [7,29]. From this point of view, metagenomic analysis based on the unique ability to identify bacteria by the presence and structure of the 16s rRNA gene has acquired leading importance. The subsequent development of whole genomic probes allows determining a fairly complete set of bacteria species included in the microbiota of periodontal pockets [54]. Due to the use of whole genome sequencing in clinical conditions, it was possible to establish the relationship of detection of *F. alocis* with the progressive course of chronic periodontitis [53].

In cases where the content of certain pathogens in the biological material is small, 16s rRNA gene sequencing is supplemented with amplification of the desired genetic material by PCR. In this way it was possible, for example, to establish the involvement of *F. alocis* not only to the development of chronic periodontitis but also to the etiology of endodontitis and periapical periodontitis [42,47].

Obtaining primers for the identification of *F. alocis* allowed us to move to a wide introduction in clinical practice of quantitative polymerase chain reaction [55]. Through the use of quantitative PCR, the conditions for the formation of microbial communities, including with the participation of *F. alocis* [3], were studied. For example, unique data were obtained on the role of the association of *F. alois* with *P. gingivalis* and *T. denticola* in the relationship of chronic periodontitis and diabetes mellitus, which leads to complicated pregnancy in patients [22].

Conclusion

The data presented above demonstrate that it was not until recently that the poorly recognizable bacterium *Filifactor alocis* was found to be a causative agent of chronic periodontitis belonging to the "red complex". Due to its pronounced protease activity and involvement in arginine metabolism, *F. alocis* colonizes the periodontal tissues and significantly affects the development of a community of periodontal microorganisms, thus contributing to the invasion of epithelial tissues by these pathogens. Furthermore, *F. alocis* resistant to oxidative stress activates production of proinflammatory cytokines, suppresses the defense reactions of neutrophilic granulocytes, and inhibits activation of the complement system. From the diagnostic point of view, the most promising method of identification of *F. alocis* in clinical practice is the polymerase chain reaction.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Declare if any financial interest or any conflict of interest exists.

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