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Taxonomy, Biology, and Periodontal Aspects of *Fusobacterium nucleatum*

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INTRODUCTION

The pathogenic potential of *Fusobacterium nucleatum* and its significance in development of periodontal diseases, as well as in infections in other organs, have gained new interest for several reasons. First, this bacterium has the potential to be pathogenic because of its number and frequency in periodontal lesions (72, 73, 114, 203, 208), its production of tissue irritants (21, 54, 218, 226, 228, 259, 269, 297), its synergism with other bacteria in mixed infections (46, 82), and its ability to form aggregates with other suspected pathogens in periodontal disease and thus act as a bridge between early and late colonizers on the tooth surface (172). Second, of the microbial species that are statistically associated with periodontal disease, *F. nucleatum* is the most common in clinical infections of other body sites (208). Third, during the past few years, cloning and sequencing and the application of new techniques such as PCR have made it possible to obtain more information about *F. nucleatum* on the genetic level, thereby also gaining better knowledge of the structure and functions of the outer membrane proteins (OMPs) (14, 15, 36, 38, 41, 145). OMPs are of great interest with respect to coaggregation (157, 158, 279), cell nutrition (26), and antibiotic susceptibility (55, 248, 271). Several studies have shown that OMPs are involved in the pathogenicity of gram-negative bacteria (32, 47, 135).

The purpose of this review is to give an overview of what is known to date about *F. nucleatum* in general, such as its taxonomy and biology, with special emphasis on its pathogenic potential. We will also focus on its possible role among other periodontal bacteria in the development of periodontal diseases, including the possible roles played by OMPs.

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F. NUCLEATUM

Taxonomy and General Characteristics

F. nucleatum is the type species of the genus *Fusobacterium*, which belongs to the family *Bacteroidaceae*. The name *Fusobacterium* has its origin in *fusus*, a spindle; and *bacterion*, a small rod: thus, a small spindle-shaped rod. The term *nucleatum* originates from the nucleated appearance frequently seen in light and electron microscope preparations owing to the presence of intracellular granules (Fig. 1) (130, 204, 238). *F. nucleatum* is nonsporeforming, nonmotile, and gram negative, with a G+C content of 27 to 28 mol% and a genome size of about 2.4×10^6 bp (34). Most cells are 5 to 10 μm long and have rather sharply pointed ends. Colony morphology is not a consistent parameter of the fusobacteria and is not sufficient for species identification (293). The bacterium is anaerobic but grows in the presence of up to 6% oxygen (204). The production of butyric acid as a major product of the fermentation of glucose and peptone, together with characteristic lipid constituents, differentiates *Fusobacterium* species from other anaerobic, gram-negative, nonsporeforming rods. *F. nucleatum* has no sialidase activity (198).

The species *F. nucleatum* is considered to be rather heterogeneous. On the bases of electrophoretic patterns of whole-cell proteins and DNA homology, Dzink et al. (70) have proposed dividing *F. nucleatum* into three (or four) different subspecies: subspecies *nucleatum*, *polymorphum*, and *vincentii*. On the bases of DNA-DNA hybridization patterns and electrophoretic patterns of the enzymes glutamine dehydrogenase and 2-oxoglutarate reductase, Gharbia and Shah (95, 96, 98) divided *Fusobacterium* species into four subspecies: subspecies *nucleatum*, *polymorphum*, *fusiforme*, and *animalis*. Strain ATCC 25586 is the type strain of *F. nucleatum* subsp. *nucleatum*, and ATCC 10953 is the type strain of *F. nucleatum* subsp. *polymorphum*. Heterogeneity within *F. nucleatum* is also reflected in the DNA methylation pattern (37).

It is widely recognized that comparative analysis of small-subunit rRNA gene sequences currently represents the most powerful method for investigating the natural supraspecific interrelationship of microorganisms (220, 316). Intrageneric relationships of members of the genus *Fusobacterium* have

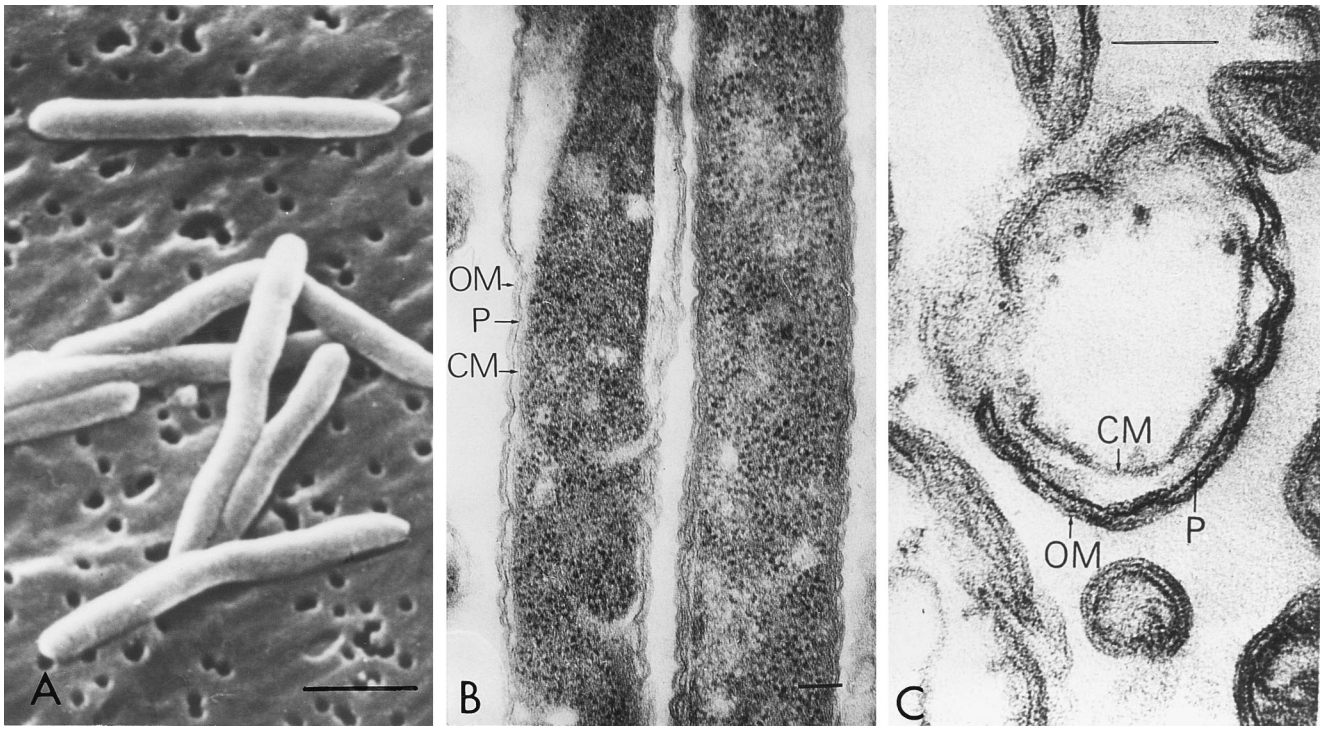

FIG. 1. Electron microscopy (EM) of *F. nucleatum* Fev1. (A) Scanning EM. Bar, 1 μm. (Kindly provided by Karl A. Brokstad.) (B and C) Transmission EM showing sections through intact cells (B) and French pressed cells (C). Bars, 100 nm. Panels B and C are reprinted from reference 18 with permission of the publisher. OM, outer membrane; P, periplasmic space; CM, cell membrane.

been determined by reverse transcriptase sequencing of small-subunit rRNA (178, 179). The subspecies of *F. nucleatum* (subspecies *nucleatum*, *polymorphum*, *fusiforme*, and *animalis*) and *F. alocis*, *F. periodonticum*, and *F. simiae*, which colonize oral cavities, exhibited high levels of sequence homology with each other and formed a distinct group within the genus. Although all of the *Fusobacterium* strains tested had unique rRNA gene sequences (approximately 1,300 bases were examined), the four subspecies of *F. nucleatum* exhibited relatively high levels of sequence similarity (97.3 to 98.4%). Particularly noteworthy was the exceedingly high level of sequence relatedness (99.5%) between *F. nucleatum* subsp. *nucleatum* (ATCC 25586) and *F. periodonticum*. On the other hand, variable results have been reported from DNA-DNA hybridization studies of these two species: from 7% (186), to 38% (229, 261), to 63 to 76% homology (179). The *F. nucleatum* subspecies and *F. periodonticum* show a high level of phenotypic resemblance (261); they produce indole in peptone-containing medium (204, 261, 293), ferment glutamine via the 2-oxoglutarate pathway, and contain a peptidoglycan based on *meso*-lanthionine (301, 303), and their growth is usually inhibited in the presence of bile (293). They both carry the fatty acid 3-hydroxyhexadecanoate (3-OH-16:0) as a distinctive characteristic, although they differ somewhat in fatty acid methyl ester patterns (143, 293). The two species have similar G+C contents (186, 229). This close relationship between *F. nucleatum* and *F. periodonticum* was also found to include the presence of a 40-kDa major OMP, the FomA porin (35). By phylogenetic grouping through oligonucleotide analysis of the 16S rRNAs, *F. nucleatum* was found to be closely related to *Bacteroides* spp. and the flavobacteria (225, 308; see also Woese et al. [317]), and some similarity has also been found in the DNA (39) and antigenic compositions (74) of these species. However, care should be taken when claiming phylogenetic relationship. Differences in gene arrangements may be more important in defining the identity of a bacterium than the differences in either nucleotide sequences of structural genes or amino acid sequences of proteins (234). In recent studies based on the 16S rRNAs, *F. nucleatum* and *Bacteroides* spp. are placed into two different phyla (220, 283). When antisera to the *Escherichia coli* RNA polymerase core enzyme and sigma factors have been used to examine the RNA polymerase of *F. nucleatum*, they have been found to differ in antigenicity (162).

Although there are some observations indicating piluslike fimbriae in *F. nucleatum* (118, 154), more recent studies have not verified this phenomenon, suggesting that this bacterium does not possess fimbriae, pili, or flagellae (18, 60, 78, 120, 157). It occasionally has a mucopolysaccharide capsule of variable thickness, which may be important for its pathogenic capability (45, 46).

F. nucleatum possesses an outer membrane characteristic of gram-negative bacteria (11, 15, 18, 187, 302). The cell envelope consists of outer and inner (cytoplasmic) membranes separated by a periplasmic space containing the peptidoglycan layer (18). In general, in gram-negative bacteria the inner membrane constitutes a symmetrical phospholipid bilayer with phospholipids and proteins present in about equal amounts. The outer membrane functions as a molecular sieve and is an asymmetric membrane consisting of phospholipids, lipopolysaccharides (LPS), lipoproteins, and proteins. About one-third of the mass of the fusobacterial outer membrane is proteins, and these form a characteristic protein profile upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (18). Some of the proteins are highly expressed pore-forming units (Fig. 2) (15, 18, 216, 279) (see below).

Analysis of patterns of cellular fatty acids in *Fusobacterium*

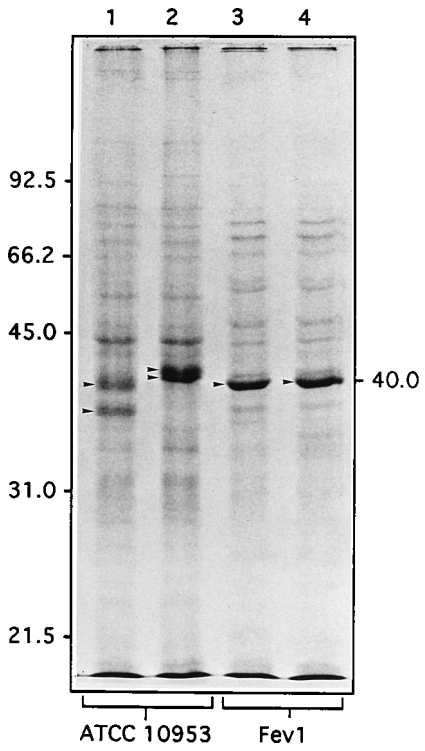

FIG. 2. Protein profiles of the OMPs of *F. nucleatum* ATCC 10953 and Fev1. The samples were heated at 50°C (lanes 1 and 3) and 100°C (lanes 2 and 4) before SDS-PAGE analysis. Molecular mass standards (in kilodaltons) are indicated on the left. Proteins with changed mobility and the 40-kDa non-heat-modifiable OMP of Fev1 are indicated by arrowheads. Reprinted from reference 18 with permission of the publisher.

species has been used as a tool for identification to the species level (293). As mentioned, Jantzen and Hofstad (143) and Tunér et al. (293) found *F. nucleatum* to contain 3-OH-16:0 as a distinctive character, whereas Calhoon et al. (49) found small quantities of this fatty acid in other fusobacterial species as well. This 3-OH-16:0 acid is a group-specific constituent of LPS in *F. nucleatum* (123, 132). LPS from oral strains of *F. nucleatum* consist of a typical lipid A component, exhibiting a close structural relationship to that of other groups of gram-negative bacteria (123), and an O-antigen heteropolysaccharide (133, 174) and resemble LPS of other gram-negative bacteria (60). The LPS of *F. nucleatum* contain 3-deoxy-D-manno-octulosonic acid (KDO) (84, 131, 132). *F. nucleatum* strains have been classified into six chemotypes on the basis of the polysaccharide composition of their LPS (84). Typical sugar constituents are glucosamine, glucose, 3-deoxy-D-manno-octulosonic acid, and L-glycero-D-manno-heptose. Some strains also contain D-glycero-D-manno-heptose, galactose, and rhamnose. In addition to the heptoses, LPS from *F. nucleatum* also contain significant amounts of β-hydroxymyristic acid. A common core epitope has been found in LPS preparations from *Eikenella corrodens* and *F. nucleatum* ATCC 25586, using monoclonal antibodies (149, 150). *F. nucleatum* LPS are endotoxins (277, 278) and possess O-antigenic specificity (133, 174). They belong to the enterobacterium-type LPS and have been found to possess biological activities comparable to those of LPS of certain strains of *E. coli* in terms of activation of *Limulus* lysate, local Schwartzman reaction, B-cell mitogenicity, polyclonal B-cell activation, induction of bone resorption, and interleukin-1 (IL-1) production by macrophages (117, 134). The cationic polypeptide antibiotic polymyxin B was found to abrogate the mitogenic activity of LPS from *F. nucleatum* and *E. coli*, presumably due to the formation of a polymyxin B-LPS complex which reduces mitogenic activity. Complement activation (C3) has been demonstrated by LPS purified from *F. nucleatum* isolated from infected root canals, suggesting an inflammation-provoking ability (137).

Lanthionine has been found to be a natural constituent of the peptidoglycan of *F. nucleatum* and some other fusobacterial species and replaces *meso*-diaminopimelic acid, which normally is present in the peptidoglycan layer in gram-negative bacteria (83, 147, 148, 300, 301, 303). Human lysozyme is capable of dissolving the peptidoglycan layer of *F. nucleatum* (302).

Occurrence and Role in Periodontal Diseases

F. nucleatum is one of the most common species in human infections and can be found in body cavities of humans and other animals (204, 208). Of the periodontal species that are statistically associated with periodontal disease, it is the most common in clinical infections of other body sites (208). It has been isolated from several parts of the body (25) and from infections such as tropical skin ulcers (80), peritonsillar abscesses (146), pyomyositis and septic arthritis (106), bacteremia and liver abscesses (58, 249), intrauterine infections (51), bacterial vaginosis (126), urinary tract infections (233), pericarditis and endocarditis (255, 292), and lung and pleuropulmonary infections (20, 196). The origin of *F. nucleatum* in infection has been dental in several cases (58, 106). Fusobacteria, including *F. nucleatum*, are recovered from a variety of infections in children (44).

Studies of the predominant cultivable oral microflora reveal that only a small number of the over 300 species found in human subgingival plaque are associated with periodontal disease (201, 203, 208). Collective microbiological studies implicate the gram-negative species *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *F. nucleatum*, *Capnocytophaga rectus*, *Eikenella corrodens*, *Capnocytophaga* spp., certain spirochetes, and the gram-positive *Eubacterium* spp. in adult periodontitis. *Actinobacillus actinomycetemcomitans* seems to be the prime candidate in the etiology of juvenile periodontitis (8, 53, 90, 215).

The role of *F. nucleatum* in the development of periodontal diseases has lately attracted new interest (172, 208). Of over 51,000 isolates examined by Moore and Moore (208), *F. nucleatum* and *Actinomyces naeslundii* were the most commonly occurring species in the human gingival crevice. From the early to the late stages of plaque formation, there is a shift from a gram-positive to a gram-negative microflora in which, among others, *F. nucleatum* increases in proportion as plaque forms (235). From studies on the bacteriology of experimental gingivitis in children (4 to 6 years) and young adults (22 to 31 years), *F. nucleatum* appeared to be one of the nonspirochetal organisms most closely correlated with gingivitis, and it appeared to be more common in young adults (205, 206). This also seems to be the case in naturally occurring gingivitis (140, 200). *F. nucleatum* has been detected less frequently in the first 6 months of life compared with older age groups, ranging from 25% of children below 6 months to 67% of children by 2 years, but of total anaerobic CFU, the proportion of *F. nucleatum* was generally low (85, 173). In children 5 to 7 years of age, *F. nucleatum* is found commonly in plaque, being isolated from 60 to 70% of children examined (86). Even in juvenile periodontitis lesions, *F. nucleatum* has been reported in large amounts at active sites of inflammation (8, 53, 90, 215). In a study by

Moore et al. (209), the only species that were detected in one or more samples from all subjects with active sites were *F. nucleatum*, *C. rectus*, and *Peptostreptococcus micros. F. nucleatum* is detected more commonly in dental plaque than on the tongue or in saliva, but these sites are a more common habitat of the organism than are the tonsils (86).

It has been suggested that certain combinations of bacterial species (clusters) present at the same time in the periodontal pocket are more prone to elicit periodontitis than other bacterial clusters (4, 72, 115, 265, 266, 268). In experimentally induced infections in mice, strains of *F. nucleatum* were pathogenic when administered in pure culture; however, a mixed culture of *F. nucleatum* with either *P. gingivalis* or *Prevotella intermedia* was significantly more pathogenic than *F. nucleatum* in pure culture (22). Positive correlations for disease production between *F. nucleatum*, *C. rectus*, *Prevotella intermedia*, and *Peptostreptococcus micros* have been found in periodontal as well as endodontal lesions (5, 6, 72, 73, 203, 276). Recently, Ali et al. (5, 6) demonstrated positive associations between *F. nucleatum*, *P. gingivalis*, *Prevotella intermedia*, and *B. forsythus* in subgingival plaque samples from untreated Sudanese patients with periodontitis. The most important finding was the effect exerted by *F. nucleatum* on the colonization of *Prevotella intermedia*; *Prevotella intermedia* was never detected in a site unless *F. nucleatum* also was present.

Combinations of *F. nucleatum*, *B. forsythus*, and *C. rectus* or of *P. gingivalis*, *Prevotella intermedia*, and *Streptococcus intermedius* in sites that had the most attachment loss and the deepest pockets have been reported (265). *F. nucleatum* was also present in the majority of instances when *B. forsythus* was detected (265, 266). Dzink et al. (72) detected the complex of *F. nucleatum*, *B. forsythus*, and *C. rectus* in 10 of 100 active sites, while only 2 of 150 inactive sites had this composition of organisms. The same three species were found in patients refractory to treatment and in subjects who exhibited recent alveolar bone loss (72, 265, 284). This is in agreement with the reports by Dzink et al. (73) and Tanner and Bouldin (282) which claim that *F. nucleatum* is among the bacteria most often (if not the most often) found in bacterial samples from subgingival pockets in periodontal disease, and it is also found in large amounts (114, 264). However, *F. nucleatum* is rather widespread in periodontal pockets in general, and *F. nucleatum* and *C. rectus* were the most frequently recovered species in an analysis of the subgingival flora of randomly selected subjects; 80 to 81% of the subjects were found positive for these microorganisms (224). *F. nucleatum* has been isolated from both active and inactive sites of disease, and it has been suggested that different subgroups may vary in pathogenesis and be related to different levels of disease activity (72, 73, 99, 282). The most common subspecies in the gingival crevice is *F. nucleatum* subsp. *vincentii* (this is also the case for other body sites), with *F. nucleatum* subsp. *nucleatum* and *F. nucleatum* subsp. *polymorphum* following in a ratio of 7:3:2 (208).

Growth and Metabolism

Fusobacteria require rich media for growth and usually grow well in media containing Trypticase, peptone, or yeast extract (16, 17). Much attention has been paid to the utilization of amino acids and peptides by *F. nucleatum* (16, 19, 23, 50, 54, 71, 94, 97, 100, 160, 182, 189, 226, 228, 238–240, 242, 253, 254). *F. nucleatum* seems to be one of the few nonsporulating anaerobic species that uses amino acid catabolism to provide energy, and some strains of *F. nucleatum* utilize and apparently need peptides for growth. ATCC 10953 did not use any peptides to a noticeable extent (16), whereas all other strains examined utilized peptides containing glutamate and aspartate. All strains used amino acids, and glutamate, histidine, and aspartate utilization was common to all strains. The glutamate and histidine pools were characteristically depleted before the other amino acids were attacked, and at that time all strains except ATCC 10953 started to utilize peptides at a noticeable rate. Fev1 does not grow on a medium based on amino acids alone. Most strains utilized lysine, and some strains utilized methionine, threonine, and serine, which is in agreement with the studies of Dzink and Socransky (71) and Loesche and Gibbons (182). Shah et al. (254) found *F. nucleatum* to use peptides preferentially over free amino acids. *F. nucleatum* can survive on mainly glutamate as the major energy source (97). However, whether the utilization pattern of amino acids can be related to the subclassification of *F. nucleatum* is uncertain since the results from different studies are conflicting (71, 97). Wyss (318) found phenylalanine but not aspartate to be essential for growth of *F. nucleatum*. The sweetener aspartame could be used as a source of essential phenylalanine. *F. nucleatum* is probably able to actively transport peptides into the cell (50). Enzymes involved in the fermentation of glutamate in *F. nucleatum* have been revealed (160), and *F. nucleatum* seems to be the only known gram-negative bacterium able to ferment glutamate via 2-hydroxyglutarate (48). Lysine is fermented by *F. nucleatum* with the formation of acetate and butyrate. The lysine cleavage enzyme has been purified and found to have properties much like those of the enzymes of lysine-fermenting clostridia (19). Brokstad and Jensen (43) and Brokstad et al. (42) purified and characterized a 65-kDa OMP of *F. nucleatum* which appeared to be a serine protease that might be involved in the uptake of peptides.

The utilization of peptides by these species is in accordance with available substrates in the environmental niches that these bacteria colonize. In the gingival crevice, the saccharolytic bacteria utilize the available carbohydrates. Peptides are generated by the hydrolytic activity of *P. gingivalis* (242), and therefore the levels of protein and ammonium ions are high and probably available to *F. nucleatum.*

Carbohydrate metabolism and uptake by *F. nucleatum* have been the focus of interest for several studies (59, 94, 238–241). *F. nucleatum* utilizes glucose to a low extent compared with other species, and *F. nucleatum* does not grow with sugars as the main energy source (94, 239, 241, 243). Available data on fusobacterial species indicate that glucose is used for the biosynthesis of intracellular molecules and not energy metabolism (253). The ability of *F. nucleatum* to metabolize its storage glycopolymers before utilizing amino acids has recently been demonstrated (254). *F. nucleatum* possesses an amino acid-dependent (only glutamine, lysine, and histidine are effective) carbohydrate transport system for glucose, galactose, and fructose that operates exclusively under anaerobic conditions and results in the production of polysaccharides inside the cell (238). Catabolism of these polysaccharides is controlled by the same amino acids, and the polymer can be degraded to yield butyric, lactic, formic, and acetic acids (240). Addition of glutamine, lysine, or histidine to the anaerobic cell suspension inhibits polymer degradation. Polymer catabolism is resumed when specific enzymes required for amino acid fermentation are inactivated by exposure of the cells to air (240). The energy necessary for active transport of the sugars (acetylphosphate and ATP) is derived from the anaerobic fermentation of glutamine, lysine, and histidine, and these compounds must provide the energy for glucose and galactose accumulation by a three-stage process involving membrane translocation, intracellular phosphorylation, and polymer synthesis. The capacity of *F. nucleatum* to form intracellular polymers from glucose,

galactose, and fructose under conditions of amino acid excess and to ferment this sugar reserve under conditions of amino acid deprivation (239, 241, 243) may contribute to the survival of *F. nucleatum* in the environment of the oral cavity and to the persistence of this organism in periodontal disease. Certain strains of *F. nucleatum* can catabolize dextrans, and the dextran hydrolase is found to be cell associated (59). Since dental plaque bacteria can synthesize and partly utilize dextran, it is suggested that this polysaccharide can act as a carbohydrate storage compound.

The major product from metabolism of peptone or carbohydrate by fusobacteria is butyrate without any iso-acids but often with acetate and lactate and lesser amounts of propionate, succinate, formate, and short-chained alcohols. *F. nucleatum* produces propionate from threonine but not from lactate; it does not hydrolyze esculin, but it produces indole. Butyrate, propionate, and ammonium ions inhibit proliferation of human gingival fibroblasts (21), may have the ability to penetrate the gingival epithelium (259), and are present in elevated levels in plaque associated with periodontitis. Because of this, they may have an etiological role in periodontal disease. Although the effect of the metabolites is not sufficient to cause cell death, inhibition of fibroblast proliferation is serious because the potential for rapid wound healing is compromised. Proteases from pathogenic bacteria can act as direct proteolytic activators of human procollagenases and degrade collagen fragments. Thus, in concert with host enzymes, the bacterial proteases may participate in periodontal destruction (269). *F. nucleatum* is capable of desulfuration of cysteine and methionine, resulting in the formation of ammonia, hydrogen sulfide, butyric acid, and methyl mercaptan (54, 226, 228). Hydrogen sulfide and methyl mercaptan account for 90% of the total content of volatile sulfur compounds in mouth air (291). A biotin-dependent sodium ion pump from *F. nucleatum*, glutaconyl-coenzyme A decarboxylase, has been characterized (23). The decarboxylation of glutaconyl-coenzyme A to crotonyl-coenzyme A is a key step in the fermentation of glutamate to acetate and butyrate by bacteria via the hydroxyglutarate pathway. Studies on enzymatic activities that may be involved in the formation of sulfur compounds from cysteine and methionine have shown that multiple forms of enzymes are involved, and in *F. nucleatum* multiple forms of L-cysteine desulfhydrase activity have been confirmed (54). The presence of γ-glutamylpeptidases is very characteristic of *F. nucleatum* strains, and it appears that the enzymes are associated with the regulatory functions of glutathione (189).

From a nutritional point of view, the organization of different bacterial species, for example, saccharolytic and asaccharolytic species, aerobic and anaerobic species, and clusters of bacteria in the tooth environment, is fascinating and logical (111). There exists a symbiotic life in the periodontal pocket that apparently several species make use of. This is best illustrated by the coexistence of different bacterial species in clusters and by coaggregation of *F. nucleatum* and *P. gingivalis* in intimate contact, which probably supplies each with essential metabolites. The saccharolytic aerobic bacteria found mostly in supragingival plaque convert carbohydrates into short-chain organic acids, lowering the pH in the local environment. The asaccharolytic bacteria are nearly always anaerobic and generally found subgingivally, where they utilize nitrogenous substances for energy, are usually weakly fermentative, and tend to raise the local pH (253). More than 90% of the carbohydrates utilized by bacteria in dental plaque are used for energy production (122), but carbohydrates are also utilized by asaccharolytic species like *F. nucleatum* in which, e.g., glucose is used for biosynthesis of intracellular macromolecules and not energy metabolism (94, 253). Most of the carbohydrate utilized by the subgingival microflora is probably derived from the carbohydrate side chains of glycoproteins. Removal of the carbohydrate residues leaves the protein core available for further hydrolysis by the asaccharolytic species. As reported by Shah and Gharbia (253), protein hydrolysates are important as high-energy growth substrates. Peptone and tryptone proved to be excellent substrates for growth of *F. nucleatum*, whereas Casamino Acids generally were poor substrates, reflecting the need for peptides as well as free amino acids for growth. *F. nucleatum*, however, has a greater capacity to ferment free amino acids than do black-pigmented bacteroides (253).

Immunological Aspects

The nature and contribution of the immune system to the pathogenesis and etiology of periodontal disease are unclear. Several studies provide evidence for either a protective or a destructive role or a combination of the two (257). Although the leukocyte population found in inflamed gingiva is comparable to that found in reactive lymph nodes, with a predominance of B lymphocytes and $CD4^+$ (T4) helper T lymphocytes, the protective effects seen in organized lymphoid tissues are not similar in connective tissues such as gingiva and often result in a state of hypersensitivity that clinically appears injurious.

Higher serum antibody titers to *F. nucleatum* have been reported in patients with periodontitis than in patients with gingivitis or healthy individuals (61, 113, 176, 214, 287, 289, 304, 305). The serum immunoglobulin G antibody level to *F. nucleatum* is positively associated with the increase in inflammation during the first 3 weeks of an experimental gingivitis (61). Although many organisms of the subgingival flora elicit antibody responses (114, 287), the bacterium and antibodies directed against it are not always present at the same time (114, 312). This suggests that in some instances periodontal infections may be sequential and/or that a protective immunity against reinfection by the same microorganism is established. Shenker (257) proposed a model of immunologic dysfunction that occurs in the earliest stages of periodontal disease progression and is followed by a period of delayed or depressed active immune reactivity (humoral and/or cellular). Early immune dysfunction may contribute to susceptibility and progression of periodontal diseases. Such a model may explain the contradictory clinical observations of the host immune response to oral pathogens and its correlation or lack of correlation with progression and severity of periodontal disease. This model is consistent with the finding that several suspected periodontal pathogens are capable of producing immunosuppressive factors (195, 257, 258). *F. nucleatum* produces factors capable of suppressing lymphocyte responses in vitro (258). Monocyte suppression of *F. nucleatum*-induced human polyclonal B-lymphocyte activation demonstrates a potent mechanism by which the host might prevent exaggerated nonspecific immunoglobulin responses when exposed to polyclonal B-lymphocyte-inducing activities of *F. nucleatum*. On the other hand, the induction of suppressive monocytes by *F. nucleatum* may result in the inhibition of host-protective immune reactions (195). Local suppression of specific antibody production by *F. nucleatum* may be the reason why Hall et al. (116) found immunoglobulins to this bacterium in supernatant fluid from juvenile periodontitis tissues in only 1 of 75 patients, even though this microorganism is often isolated from subgingival plaque of such patients and *F. nucleatum*-specific antibodies have been detected in their sera (203, 314). As discussed by Shenker (257), immunosuppression must be a relatively tem-

porary phenomenon since many patients eventually develop a detectable humoral and/or cellular immune response to periodontal infection. The reasons may be that the patients become refractory to the immunosuppressive factors; the systemic immune system eventually becomes exposed to antigens of the pathogens, with systemic activation of both cellular and humoral immune mechanisms; and a change in microbial ecology and flora may prevent bacterial strains from producing these factors. In polyclonal B-cell activation, multiple B-cell clones are stimulated, each capable of producing a specific antibody. *F. nucleatum* has such capability (27, 190–192). Interestingly, a porin fraction of *F. nucleatum* has the properties of B-cell mitogenicity and activation and macrophage stimulation (279). Polyclonal B-cell activation may have a dramatic adjuvant effect and greatly enhance antigen-induced B-lymphocyte responses in vitro (288).

Accumulation of plasma cells in chronically inflamed sites, including periodontal lesions, is common, and *F. nucleatum* antigens are capable of contributing to this effect (190). Periodontitis is an example of a chronic inflammatory disease in which the predominant cells infiltrating the lesion are of B-cell lineage (286). *F. nucleatum* has been observed to stimulate immunoglobulin G, A and M (79, 87, 113, 194, 214) and T-cell (138) responses and to activate complement (137). It has become increasingly evident that periodontitis is not only a B-cell or polymorphonuclear neutrophil (PMN) event but that both cells are involved and, perhaps equally important, antigen specific T cells are involved in control of the periodontal lesion (181, 252).

Cytokines are involved in the progress of periodontitis (29, 88, 89, 159, 306), and it appears that the matrix metalloproteinases are centrally involved in dissolution of unmineralized connective tissue and probably also in resorption of bone (30, 31, 156). Osteoclastic bone resorption appears to be initiated by removal of the osteoid layer by osteoblasts by means of a collagenase-dependent process. Cytokines, including IL-1, tissue necrosis factor alpha, and transforming growth factor α (TGF-α), are likely to regulate expression of matrix metalloproteinase genes in periodontal tissues (30). There may be an immunoregulatory imbalance in periodontal lesions. Different periodontopathic bacteria may stimulate different cell types to produce cytokines which may have synergistic or antagonistic effects. *F. nucleatum* stimulates different cell types to produce IL-1, IL-6, tissue necrosis factor alpha, and TGF-β (88, 89, 245, 306, 307) and stimulates PMNs to produce an IL-1 inhibitor (252). Cell wall products of *F. nucleatum* trigger an enhanced steady state of TGF-β mRNA production and the secretion of TGF-β by peripheral blood monocytes (306). Administration of anti-TGF-β to sites of chronic destructive inflammation blocked leukocyte recruitment and activation and also inhibited the subsequent destruction of bone and cartilage of such lesions (306). Locally produced cytokines are believed to be responsible for the bone loss and connective tissue breakdown that occur in periodontitis (251, 252, 285), while TGF-β is an important anti-inflammatory agent and IL-1 inhibitor (227). *F. nucleatum* induces mitogenic activity (69, 185, 279).

There is current evidence that cell surface components such as OMPs exhibit powerful immunobiological activities, many of which are common to those of LPS and peptidoglycans (210, 274). A serological response to cell wall proteins of *F. nucleatum* has been known for some time (129). The studies of Takada et al. (279) suggest that a porin protein of *F. nucleatum* may play a significant role in the pathogenesis of adult periodontitis. They found the bioactivities of the porin to be comparable to those of LPS, that the content of porins in the cell envelope is greater than the amount of LPS, and that the porins and the LPS may work synergistically on the processes that lead to periodontal disease. Experiments with monoclonal antibodies directed against cell surface antigens of one strain of *F. nucleatum* suggest the presence of cross-reactive antigens and some epitope sharing among different strains of *F. nucleatum* and also other fusobacterial species such as *F. russii* (28). Studies with sera from human adults with periodontitis have demonstrated antigens shared by *F. nucleatum* and *F. necrophorum* (153).

The first line of defense of the periodontal pocket is the PMNs, which make up over 90% of the leukocytes in the gingival fluid (9). Adherence of the cells is one of the earliest observable events after PMN activation. Seow et al. (250) found that *F. nucleatum* enhanced PMN adherence. This stimulating effect of *F. nucleatum* may cause release of toxic oxygen radicals and lysosomal enzymes, resulting in damage to the periodontium. *F. nucleatum* is ingested by PMNs in vitro. Kerusuo et al. (155) suggested that lectin-mediated binding plays a role in the phagocytosis of *F. nucleatum* in the absence of opsonins.

Immunization of pregnant cows with *F. nucleatum* leads to the presence of high concentrations of specific antibodies in milk (280). High-titer milk preparations have been obtained from immunized cows, and the ability of the bovine antibodies to agglutinate target bacteria has been proposed for use in oral passive immunization studies.

Susceptibility to Antibiotics

The fusobacteria are susceptible to many of the most commonly used antibiotics, but they have reduced susceptibility or may be resistant to vancomycin, neomycin, erythromycin, amoxicillin, ampicillin, and phenoxymethylpenicillin (3, 7, 112, 125, 130, 246, 294, 313). Penicillinase-producing strains of *F. nucleatum* have been isolated (112, 125, 294), and isolation of β-lactamase-producing strains of fusobacteria is increasing (142). As β-lactamase production and β-lactam resistance have been increasingly found in gram-negative bacteria, including *F. nucleatum*, the susceptibility of different bacteria to new agents has been tested (7, 270). Biapenem, imipenem, the penem WY-49605, and trospectomycin were active against *F. nucleatum* in vitro, as were the commonly used agents chloramphenicol and metronidazole.

Antimicrobial agents have been used in periodontal treatment either alone or preferentially in combination with conventional treatment to eliminate putative periodontal pathogens (262). The most extensively used antimicrobial agents as an adjunct in the treatment of periodontal disease have been the broad-spectrum bacteriostatic tetracyclines, which inhibit protein synthesis in the bacterial cells (221). Tetracycline, doxycycline, and minocycline concentrate in gingival crevicular fluid at concentrations up to five times those found in serum. As many as 75% of the bacteria in the subgingival pocket may be resistant to tetracycline after long-term, low-dose treatment (221). Besides systemic administration, antibiotics can be delivered locally to the periodontal pocket. Examples of antibiotics and antibiotic vehicles used for sustained release subgingivally are tetracycline-impregnated fibers and metronidazole gel (107, 188, 217). Tetracycline-resistant *F. nucleatum* strains have been found in subgingival plaque samples from patients with periodontal disease (237). They carry the *tetM* gene coding for proteins that protect the ribosomes from tetracycline (221). Most tetracycline resistance genes have been found on plasmids and are readily transmissible; others are located on chromosomal elements that can be transferred by conjugation (271). *F. nucleatum* strains have been shown to harbor the *tetM*

determinant on a conjugative transposon (236). Once the *tetM* determinant enters one or more strains of *F. nucleatum*, it can probably easily be transferred to other strains within the species. In addition to transferring themselves from the chromosome of a donor to the chromosome of a recipient, conjugative transposons can insert into plasmids, making them self-transmissible. Tetracycline exposure can increase resistance to other antibiotics as well, since tetracycline resistance transfer elements can carry other resistance genes (91, 271).

Abu Fanas et al. (2, 3) found that the MIC of tetracycline for gram-negative organisms like *F. nucleatum* increased after 6 weeks of use; in contrast, amoxicillin-clavulanic acid proved to be equally as effective as tetracycline but did not induce resistant strains.

OMPs of gram-negative bacteria may function as a route of entry for antibiotics, including β-lactams, tetracyclines, chloramphenicol, and hydrophilic quinolones (121, 136, 231). Studies on porin-deficient mutants of *E. coli* have revealed that hydrophilic antibiotics can traverse the outer membrane by the three porins PhoE, OmpF, and OmpC (121, 136, 211). It is likely, but has not been shown, that this is also the case with FomA of *F. nucleatum.*

Chlorhexidine, which has been shown to inhibit dental plaque formation in both short- and long-term clinical studies, is also effective against *F. nucleatum* (13).

Using a bacteriostatic antibiotic like tetracycline on indigenous pathogens is not recommended because of the increasing possibility of developing resistant strains (298). The current extensive use of tetracycline throughout the world has led to disputes over whether or not tetracycline is in danger of becoming obsolete as a clinically useful antibiotic (271). There are no data supporting the use of systemic antibiotics alone in persons with periodontitis without prior thorough mechanical debridement possibly followed by surgery. On the other hand, evidence that antibiotics can enhance the beneficial clinical effects of mechanical periodontal therapy in recurrent disease in patients who comply with good oral hygiene practices is provided in the literature (299). The combination of amoxicillin and metronidazole (mainly active against anaerobes) is among the regimens used today for periodontal therapy (141, 299).

Adhesion and Coaggregation

Bacteria adhere to host tissues by a specific interaction mediated by macromolecules on the bacterial surface that combine with complementary structures on the host cell surface (183). When bacteria adhere to each other, the phenomenon is called coaggregation, and this bacterium-bacterium interaction is defined as the recognition between surface molecules on two different bacterial cell types such that a mixed-cell aggregate is formed (163). Bacterial adherence is essential in the colonization and establishment of an infection in a susceptible host, and adherence itself is thus an important virulence factor in addition to the toxins, enzymes, and capsular substances produced by the organisms (128, 135, 197). In general, a microorganism cannot be an effective pathogen unless it adheres to and subsequently reproduces itself within a host, and adherence seems especially important in the early events of bacterial infection (56, 183).

In the oral cavity, there is a unique situation with hard tissue (the teeth) penetrating the soft tissue barrier, thereby making the periodontal pocket a niche predisposed for bacterial establishment. The direct role of bacterial adherence in the initiation of periodontal disease(s) has not been clearly demonstrated, but adhesion of oral bacteria to hard or soft tissues is of critical importance to the maintenance of bacteria in their respective econiches. Lately, efforts have been made to elucidate the role of adhesion and coaggregation in relation to periodontal disease (158, 171, 172). *F. nucleatum* participates in both adhesion and coaggregation reactions and seems to play a key role in the multigeneric coaggregation network found in the periodontal pocket (see below) (172).

Adhesins are proteins located on the surface of bacteria that mediate their attachment to specific substrates as a first step in colonization (183). These bacterial lectins appear to recognize complex oligomer polysaccharides and include fimbriae and certain OMPs on gram-negative bacteria, as well as fimbria-like appendages of gram-positive organisms. Hemagglutinins are, by definition, adhesins. *F. nucleatum* displays hemagglutination activity on sheep and human erythrocytes (62, 65, 77, 78, 139, 219, 319); attaches to human oral epithelial cells (52, 81, 103, 319), collagen (319), gingival fibroblasts, and PMNs (81, 222, 319); and shows hemolytic activity (77). The hemolytic moiety has been found in cell, cell wall, and LPS extracts (77). The binding specificity and possible bacterial receptors have been studied (76, 222). A galactose-binding protein has been suggested to be responsible for *F. nucleatum* hemagglutination (62, 76, 199). At least some of the hemagglutinins appear to be arginine sensitive, suggesting that this amino acid may function as a contact residue between bacteria and erythrocytes during agglutination (62, 281). A hemagglutinin protein isolated from *F. nucleatum* ATCC 10953 was found to be cell surface associated and appeared as a single band of about 21 kDa on SDS-PAGE gels when presolubilized in SDS at 100°C. Unboiled hemagglutinin appeared as three bands of about 21, 38, and 60 kDa on SDS-PAGE (63). Interestingly, considerable heterogeneity in the adhesive properties of various *F. nucleatum* strains has been noted (222, 319). It is known that proline-rich salivary proteins undergo conformational changes when adsorbing to surfaces such as hydroxyapatite, thereby exposing cryptitopes (hidden areas) in the molecules. It is possible that cryptitopes are involved in the attachment of *F. nucleatum* since the bacterium binds to galactosyl receptors exposed by neuraminidase (101, 102). As suggested by Gibbons (101), elevated levels of neuraminidases and proteases associated with poor oral hygiene and gingivitis may generate cryptitopes that promote colonization of gram-negative bacteria.

LPS extracts from *F. nucleatum* adhere to saliva-coated hydroxyapatite and serum-coated hydroxy beads (219). This indicates that LPS from *F. nucleatum* may play a role in adhering not only to epithelium but also to tooth surfaces, including root cement. If this is the case, it would be important to remove cement-associated contaminants, such as endotoxins, by scaling and root planing with the aim of forming new attachment.

F. nucleatum is a particularly strong activator of PMN, and the bacterium is phagocytosed and killed by the PMNs (193, 222). Direct interaction between *F. nucleatum* and PMNs can enhance PMN adherence (250). In vitro studies have shown that approximately 98% of *F. nucleatum* cells were killed during 60 min of incubation at 37°C with live PMNs (193). Addition of GalNAc to the suspension completely inhibited killing of the fusobacteria. Lectinlike interactions between *F. nucleatum* and PMNs are probably mediated by the fusobacterial cell wall proteins previously reported to mediate binding of *F. nucleatum* to human erythrocytes, epithelial cells, fibroblasts, and lymphocytes (193, 295). Such interactions between PMNs and *F. nucleatum* should occur in vivo because *F. nucleatum* is one of the most common bacteria present in subgingival plaque (72, 73, 263, 265, 266), and PMNs compose the highest percentage of inflammatory cells in the gingival sulcus (223). The avid adherence of *F. nucleatum* to PMNs can occur in the

absence of serum opsonins, such as antibodies and complement, and may be associated with pathogenesis of periodontal disease since the interactions induce release of phlogistic products from PMNs, such as superoxide anions and lysosomal enzymes, which damage host tissues. *F. nucleatum* adheres to and activates human lymphocytes apparently by both lectinlike interactions inhibited by GalNAc and non-lectinlike interactions (295, 296).

Fibronectin is a large glycoprotein found in the extracellular matrix of loose connective tissue, in plasma, and in saliva (139). *F. nucleatum* shows strong fibronectin-binding capacity (139). The biological significance of binding of bacteria to fibronectin is unclear. It has been suggested that fibronectin mediates the adhesion of bacteria to eukaryotic cells (1), but while epithelial cells recognizing the gram-positive bacteria were rich in fibronectin, epithelial cells recognizing the gram-negative cells were lacking fibronectin. It seems that hemagglutination activity does not relate to fibronectin-binding capacity (139).

The basement membrane, located between the sulcular epithelium and the subjacent connective tissues, is the last potential barrier to bacterial translocation from the pocket into the connective tissues (109, 272). However, the epithelial lining of periodontal pockets has tunnels or holes that may provide the bacteria with portals of entry into adjacent periodontal tissues. The abilities of bacteria to adhere to and degrade basement membranes in vivo should be considered important steps for the potential active and passive invasion of gingival tissues. That *F. nucleatum* binds in high numbers to basement-membrane-like matrices in vitro and to type 4 collagen is of interest (315, 319).

F. nucleatum binds to the galactose termini of a glycosylated proline-rich glycoprotein in the parotid saliva, and deglycosylation of this purified glycoprotein results in loss of receptor activity (104, 230). A mutation in the gene encoding this salivary protein inhibits the ability to interact with *F. nucleatum* (10). Such interactions between salivary glycoproteins and *F. nucleatum* may be important in individual differences in establishing the intraoral ecology and in susceptibility to clinical disease (10, 64). As mentioned, *F. nucleatum* has proven to bind significantly to galactose in vitro (213). Galactose-binding lectins with apparent molecular weights of 300,000 to 330,000 and about 40,000 adhere to galactosyl residues on, for instance, saliva-coated surfaces or *P. gingivalis* and *Selenomonas* spp. (76, 157, 158, 166, 168, 212, 213). In addition to the lectinlike, sugar-sensitive (GalNAc) adhesins that require Ca^{2+} for activity, others are amino acid sensitive (arginine), require no divalent cation, and are trypsin and pronase P resistant, while still others are resistant to both arginine and GalNAc (222). The complexity of the binding specificity of bacterial adherence may reflect the specific localization of bacteria and the establishment of complex microflora.

Coaggregation is a phenomenon prevalent among oral bacteria isolated from the human oral cavity. There is surprisingly little or no evidence for coaggregation among resident bacteria in other ecosystems (163). Coaggregation is a direct bacterium-bacterium interaction and is highly specific in that only certain cell types are partners. The interactions are usually mediated by lectin-carbohydrate molecules on the partners and are not caused by soluble molecules or suspended substances (163). Since viable as well as dead cells coaggregate, the interactions must depend on existing surface molecules and not on a response by viable cells (163). The recognition may be intrageneric, intergeneric, or multigeneric in nature (163, 164, 165, 169), and in all three kinds of coaggregations the cells appear to interact independently of other cells in the population (165).

Surprisingly, intrageneric coaggregation among strains of oral bacteria is found infrequently and seems to occur only among the early colonizers of the tooth, i.e., most streptococci and some *Actinomyces* species (169). This may explain the dominance of streptococci as primary colonizers on cleaned tooth surfaces. The fusobacteria, which coaggregate with the widest range of genera tested so far, do not coaggregate with other fusobacteria (168). Intergeneric coaggregation is defined as cell-to-cell recognition and adherence between bacterial pairs from different genera. Fusobacteria are not motile so they may rely on cell-to-cell contacts to provide a necessary metabolic environment (168, 171). *F. nucleatum* also participates in multigeneric coaggregation, i.e., interacting bacterial networks composed of coaggregating cells of three or more genera (164, 165). The multigeneric aggregations are characterized by the stability of coaggregation partners, the independent nature of interactions, and partner specificity (165). By multigeneric aggregation, the noncoaggregating cell types are bridged together by a common partner. If one of the cell types is in a 10-fold or greater excess, morphological shapes of corncobs and rosettes can be formed (66, 177). Fusobacteria coaggregate with some strains of all genera tested so far, but in contrast to many other bacteria participating in coaggregation, each strain of *F. nucleatum* coaggregates only with a certain set of partners, indicating that recognition of partner cell surface is selective (164).

It is proposed that fusobacteria act as a bridge between early and late colonizers. The early colonizers adhere to the tooth pellicle and coaggregate with other early colonizers and also with *F. nucleatum*. Late colonizers, such as *Selenomonas flueggi*, *P. gingivalis*, and species of *Eubacterium*, *Actinobacillus*, *Capnocytophaga*, and *Treponema*, coaggregate almost exclusively with *F. nucleatum*, which seems to play a very important role by bridging these coaggregations with early colonizers (168, 170–172). The late colonizers either do not adhere to saliva-coated hydroxyapatite or adhere nonspecifically.

Several adhesins from gram-negative and gram-positive bacteria have now been purified, and some of them have been cloned. The adhesins are different kinds of proteins, and galactosides appear to be the sugar moiety most commonly recognized by oral bacterial lectins. Some appear to be fimbria-associated proteins, while others appear to be part of the outer membrane of the bacteria, as is probably the case with *F. nucleatum* adhesins (67, 151, 152, 168). Lately, several OMPs of approximately 40 kDa have been the focus of interest (15, 36, 41, 152, 158, 279). The 39.5-kDa major OMP isolated from ATCC 10953 by Kaufman and DiRienzo (152) may be similar to the 40-kDa OMP we have studied (15, 36, 41) as well as to the 41-kDa porin studied by Takada et al. (279). Kaufman and DiRienzo (152) proposed that the 39.5-kDa OMP is a receptor polypeptide participating in fusobacterial corncob coaggregations. This would be in line with the dual role often played by porins. However, there is evidence for the existence of more than one class of corncob receptor in *F. nucleatum* (151). Attachment of streptococci to fusobacteria can be mediated through fimbriae localized on the surface of the cocci (120, 124, 177). From studies of DiRienzo et al. (66), it appears that at least two types of corncob receptors are involved in binding of *F. nucleatum* to *Streptococcus sanguis*, one lipoteichoic acid-binding protein that is a loosely bound surface protein in *F. nucleatum* (67) and one that does not bind lipoteichoic acid, which is exposed on the cell surface and is firmly anchored in the outer membrane (151, 152). Kinder and Holt (157, 158) have isolated a 42-kDa *F. nucleatum* T18 OMP. The adhesin activity of *F. nucleatum* T18 from a monkey was localized to this protein, which mediated the interaction to *P. gingivalis* (158, 163). The *F. nucleatum* ATCC 10953 adhesin involved in

coaggregation with *S. sanguis* CC5A was found to be trypsin sensitive, whereas the adhesin of *F. nucleatum* T18 involved in coaggregation with *P. gingivalis* T22 is trypsin resistant. This suggests that although these coaggregations appear to be mediated at least in part by the same protein, the interactions most likely involve distinct domains, one resistant and one susceptible to trypsin treatment.

F. nucleatum coaggregates with *P. gingivalis* via a galactose-containing carbohydrate on *P. gingivalis* and an OMP from *F. nucleatum* (157, 158, 166). While numerous partners have been reported in coaggregations with *F. nucleatum*, Kolenbrander et al. (167, 168) primarily found *F. nucleatum* to coaggregate with *P. gingivalis*. It is therefore likely that *F. nucleatum* plays an important role in the establishment of *P. gingivalis* in the periodontal pocket, and the coaggregation may be a prerequisite for a successful colonization by *P. gingivalis*, whose numbers are elevated in plaque samples taken from sites exhibiting active destructive periodontal disease. In contrast to *F. nucleatum*, which is found in both healthy and diseased sites, *P. gingivalis* is found primarily in patients with periodontal disease. *F. nucleatum* is the most frequently detected species in active disease sites (72). Since fusobacteria accumulate glucose in the form of intracellular glucan, which can be used as an energy source when glucose becomes a limiting nutrient, it is possible that small amounts of glucose are excreted from the bacterial cell. This would encourage other bacteria to localize near the surface of the fusobacteria and encourage subsequent attachment (171). Since *F. nucleatum* colonizes habitats in which amino acids and peptides are their main energy source and shows no or weak intrinsic proteolytic activity (for references, see reference 42), it will profit from the coexistence with other species that produce proteolytic enzymes and release peptides needed for fusobacterial growth. In the presence of the proteolytic species *P. gingivalis*, the limited capacity of *Fusobacterium* spp. to hydrolyze proteins appeared to increase by approximately 30%, and the combination may represent a type of bacterial interaction in which peptides may become available to *Fusobacterium* species in vivo (100). It should be mentioned that *P. gingivalis* may also coaggregate with *Treponema denticola* and some strains of *S. sanguis* and may coadhere with *Actinomyces viscosus* (75, 110, 127, 180, 260).

F. nucleatum coaggregates with *Candida albicans* via bacterial cell surface proteins and carbohydrate residues on the yeast cell surfaces (12). *Fusobacterium* is the only genus tested to date that coaggregates with the gram-positive anaerobic rod *Eubacterium*. The coaggregation between these species is probably mediated by a protein-protein interaction (92).

Coaggregations with a wide variety of partners may play an important role in the maintenance of fusobacteria in the oral cavity, considering the fact that fusobacteria adhere very poorly to human cheek epithelial cells. Consequently, studies of fusobacterial OMPs are of interest to understand such interactions and important biological functions.

OMPs

It is assumed that components of the outer membrane, among which are proteins, are involved in the pathogenesis of infection of gram-negative bacteria (47). The OMPs can function as receptors for phages and mitogens (231, 232) and act as specific substrate binding sites. Some OMPs are pores that are important for transport of nutrients (119, 216, 244), and some are involved in coaggregation between bacteria (67, 151, 152, 158, 163, 168). OMPs may serve as antigens and have been considered candidates for the production of vaccines (14, 33, 47, 105). It has been demonstrated that OMPs of gram-negative bacteria can be involved in invasion of tissue (65, 232) and that surface-exposed loops of OMPs are of importance for virulence (24).

During the last decade, a few OMPs of *F. nucleatum* have been identified and characterized in some molecular detail (Fig. 2) (14, 15, 18, 36, 41, 42). The N-terminal amino acid sequence of a major 40-kDa OMP, FomA, of some strains of *F. nucleatum* was obtained by Bakken et al. (15), and this OMP has been the focus of special interest (67, 157, 158, 279). The gene *fomA* encoding the FomA porin monomer was sequenced by Bolstad et al. (36, 41) for *F. nucleatum* subsp. *nucleatum* type strain ATCC 25586, *F. nucleatum* subsp. *polymorphum* type strain ATCC 10953, *F. nucleatum* Fev1, and *F. periodonticum* ATCC 33693 (35, 36, 41).

It has now been shown that FomA is a porin (161). The exact β-barrel structure of porins was first determined for the *R. capsulatus* porin (309, 310). A topology model of the porin PhoE of *E. coli* was proposed by Tommassen (290), and this was found by X-ray crystallography to be correct (57). The functional unit of these porins appears to be a trimer. The polypeptide chain of each monomer traverses the outer membrane 16 times, mostly as amphipathic β-strands, and thereby exposes eight regions to the cell surface, most of which are highly variable. One of the eight loops seems to extend into the interior of the pore, where it constricts the channel and seems to be important in determining the channel size (275). The porins do not contain hydrophobic segments long enough to span the outer membrane. The membrane-spanning regions contain alternating hydrophilic and hydrophobic segments resulting in amphipathic strands, which constitute an integral structure in the hydrophobic outer membrane. By making barrels with apolar external surfaces and hydrophilic internal surfaces, they can exist in the lipid environment (57, 144). The porins form hydrophilic channels with some ion selectivity (216). Recently, it has been demonstrated that FomA is a general nonspecific porin that is present in the outer membrane as a trimer (161). The FomA porins have weak ion selectivities that apparently depend on the overall charges of the monomers. By applying rules developed from the general characteristics of the well-studied *E. coli* OMPs (290), topology models were made for the 40-kDa OMPs of the three *F. nucleatum* strains (41) and the *F. periodonticum* strain mentioned above (35) (Fig. 3). The fusobacterium models fit very well with the requirements for porin structures. The hydropathy profiles of OMPs of all FomA proteins are similar. The eight loops that we presume are surface exposed represent hydrophilic maxima, while the membrane-spanning segments that probably traverse the membrane as amphipathic β-strands represent hydrophobic maxima. The great variability of the proposed surface-exposed loops of the strains examined may reflect the enormous pressure from other organisms and the host's defense mechanisms to which the bacteria are exposed in the periodontal pocket. There is probably a "war" going on in the periodontal pocket in which the capability of changing the surface-exposed loops and epitopes over time would be an effective defense mechanism that would enable the bacteria to avoid specific bacterial competitors and immunological mechanisms. The structure and function and the structure-function relationship of the 40-kDa porin of *F. nucleatum* are under investigation.

Some of the DNA probes available for *F. nucleatum* cross-react with other bacterial species (68, 175, 308). Specific DNA probes have been made from the conserved parts of the *fomA* sequence and also by random cloning of DNA fragments (38, 40). Interestingly, AGA is the only codon used for arginine in

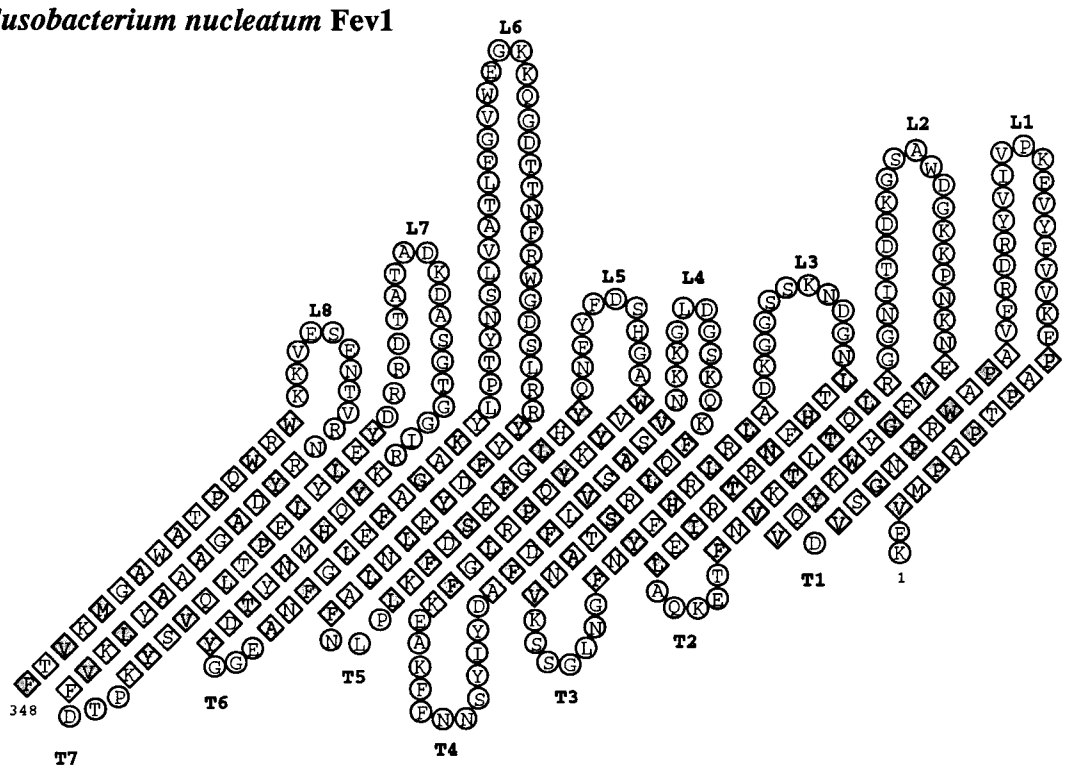

FIG. 3. Topology model of the FomA protein monomer of *F. nucleatum* Fev1 with amino acid sequence in one-letter code. The view is from the 16-stranded antiparallel β-barrel, which is unrolled. The top part of each model shows the surface-exposed regions, whereas the central part indicates the presumed transmembrane segments. Amino acid residues are indicated by diamonds where they are supposed to form a β-strand (shaded if their side chain is supposed to be external, i.e., directed towards the lipids, or the subunit interface) and by circles for turns (T) and loops (L). Reprinted from reference 41 with permission of the publisher.

the *fomA* gene of all tested strains. In contrast, this codon is rarely used in *E. coli* (256).

Pathogenic Potential in Periodontal Diseases

There are many difficulties in the search for the etiologic agents of destructive periodontal diseases. These difficulties include technical problems, such as acquiring an appropriate microbial sample, but also difficulties in determining the state of activity of periodontal disease (267). It has been shown that sampling procedure alone could explain up to 98% of false-negative results when the influence of sampling procedure on the recovery of bacteria from periodontitis sites was evaluated (311). Another level of complexity exists in that strains within species differ in virulence, as has been suggested for *F. nucleatum* subspecies (90, 99). The use of gene probes to identify bacteria has improved the chance of obtaining a more correct picture of which bacteria are present in the periodontal pocket at the time of examination. In addition, new methods for quantitating the bacteria, such as measurement of the strength of radioactive or chemiluminescent signals from colony blots and quantitative PCR, are now available.

Nevertheless, *F. nucleatum* is known to have the potential to be a periodontal pathogen. One important feature is the production of toxic metabolites. Comparison of direct cytotoxicity for human gingival fibroblasts of sonic extracts from several periodontal bacteria has shown that the sonic extracts from *F. nucleatum* and *A. actinomycetemcomitans* consistently appear to have the most profound effects at the lowest concentrations tested (273). The ability of components of these organisms to kill or arrest the proliferation of the normal resident cells of the periodontium (the fibroblasts) must play a role in their ability to produce disease (184, 185, 273). The formation of sulfides by the microflora may provide a way for the bacteria to escape important parts of the host immune system, and it is probable that the sulfide interferes with opsonization of the bacteria (108). Butyrate, propionate, and ammonium ions, which are produced by *F. nucleatum*, inhibit proliferation of human gingival fibroblasts, may have the ability to penetrate the gingival epithelium, and are present in elevated levels in plaque associated with periodontitis. Therefore, they may have an etiological role in periodontal disease (21, 259). Although it seems that the effect of the metabolites is not toxic to the point of causing cell death, the inhibition of fibroblast proliferation is severe because the potential for rapid wound healing is compromised. In addition to producing toxic metabolites, *F. nucleatum* has the ability to adhere to and degrade basement membranes in vivo and to bind to type 4 collagen (315, 319). Acting together with other periodontal bacteria, it has synergistic effects, and it possesses major OMPs that may be important for virulence. A porin fraction of *F. nucleatum* has properties of B-cell mitogenicity and activation and macrophage stimulation (279).

Because of the numbers and frequency of *F. nucleatum* and *Eubacterium nodatum* and their production of butyric acid as a tissue irritant, Moore et al. (209) suggested that these organisms should be prime suspects in the initiation of periodontal disease and that several other species may contribute to tissue destruction once gingival irritation has been produced (208). "Colonization of teeth with actinomyces and streptococci which coaggregate with *F. nucleatum* and other species to produce tissue irritation, bleeding, and serum exudate that

stimulate species of *Porphyromonas* and *Prevotella* and a number of other species associated with overt tissue destruction'' is proposed by Moore et al. (209) as a possible theory for development of periodontal disease. Socransky et al. (266) proposed that as *F. nucleatum* provides essential growth requirements for *B. forsythus*, this combination, possibly with *C. rectus* or other species, might be necessary to induce disease. It is also possible that even though the bacteria could be pathogenic individually, their combination could produce synergistic or additive damage to the periodontal tissues such as has been proposed for *P. gingivalis* and *F. nucleatum* (45, 46, 82).

SUMMARY AND CONCLUSIONS

In summary, *F. nucleatum* may constitute a considerable part of the subgingival flora of gingivitis in children and adults and of periodontitis in juveniles and adults. It is present in larger amounts in adults than in children and in diseased sites than in healthy sites (200, 202–204, 206–208, 247). *F. nucleatum* exhibits several biological activities related to the etiology of gingival inflammation and oral diseases: the fusobacteria have the ability to participate in a broad range of coaggregations, they are among the most frequently isolated bacteria in plaque from healthy sites, their numbers increase about 10-fold in plaque samples from periodontally diseased sites, and they are in general the most frequently isolated bacteria in disease (208). *F. nucleatum* plays an important role also in serious infections in other parts of the body. An accurate identification of fusobacterial species is therefore of great importance not only for taxonomic reasons but also for appropriate treatment of infection, since the susceptibility of different fusobacterial species to antibiotics varies widely (93). The most rapid and specific means of identification of *F. nucleatum* appears to be by DNA or rRNA probes which have been developed during the last few years.

In closing, it must be emphasized that although much has been learned of the involvement of *F. nucleatum* in the infection process, very little is known of the exact reactions taking place. The etiology of periodontal disease is complex and multifactoral, the fundamental factors being the bacteria and the immune system. There are reasons to believe that further studies may help to elucidate more of this still misty area; in particular, further studies of functions and structure-function relations of *F. nucleatum* OMPs may contribute significantly to progress. The cloning and expression of proteins from mutated strains should be of great importance in this respect.

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