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Serological Responses to Antigens of *Bacteroidaceae*

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INTRODUCTION

The family *Bacteroidaceae* comprises obligately anaerobic, nonsporeforming, gram-negative rods classified into the genera *Bacteroides*, *Fusobacterium*, and *Leptotrichia*. Their habitat is the mucous membranes of humans and animals. In the lower intestinal tract they are the predominant microbial form.

This review will discuss the serology of this important group of bacteria—in particular, studies carried out during recent years. Most of the work in this field has been done on the species isolated most often from anaerobic infections in man, viz., the *B. fragilis* group of organisms (9), *B. melaninogenicus*, and *F. nucleatum*. Several studies have also been performed on *F. necrophorum* and *B. nodosus*, which are important animal pathogens (85, 109).

ANTIGENS ISOLATED FROM *BACTEROIDACEAE*

Cell Wall Lipopolysaccharides

Preparation. Extractions with aqueous phenol (125) have been used for extraction of lipopolysaccharides (LPS) from *F. nucleatum* (17, 34, 63, 78, 94), *F. necrophorum* (36, 56, 90, 121), *L. buccalis* (17, 42, 70), *B. fragilis* (51, 54, 91, 92), *B. melaninogenicus* (46, 55, 70), *B. oralis* (14), and some other *Fusobacterium* and *Bacteroides* species (48). In most instances the LPS were purified from the water phase by ultracentrifugation. Knox and Parker (70) purified their LPS from the phenol phase. As an alternative to preparative ultracentrifugation, Hofstad (51) and Hofstad and Kristoffersen (55) tried to purify *B. fragilis* and *B. melaninogenicus* LPS from the water phase and from the supernatant fluid after ultracentrifugation of the water phase by different forms of chromatography.

Kasper and Seiler (68) used treatment of whole bacterial cells with ethylenediaminetetraacetate for extraction of LPS from *B. fragilis*. The LPS was separated from other cell membrane components by chromatography through Sephadex G-100, using a disaggregating buffer for elution. The same method was used by Mansheim and Kasper (88) for isolation of LPS from *B. asaccharolyticus* (syn. *B. melaninogenicus* subsp. *asaccharolyticus* [31]). Hofstad et al. (58) tried trichloroacetic acid and also ethylenediaminetetraacetic acid for extraction of LPS from *B. fragilis*, *B. melaninogenicus*, and *B. oralis*.

Electron microscopy of the LPS from *F. nucleatum*, *F. necrophorum*, *B. melaninogenicus*, and *B. fragilis* revealed a variety of particles differing in shape and size, such as doughnut- or disk-shaped particles delimited by a dense line or a triple-layered surface, straight or curved

TABLE 1. *Antigens isolated from Bacteroidaceae*

Antigen	Chemical nature	Microorganism	Mode of reaction	References
Capsular	Polysaccharide	*B. melaninogenicus*	Whole cell agglutination	118, 119
Surface layer	Polysaccharide	*B. fragilis*	Radioactive antigen-binding assay, precipitation, indirect immunofluorescence	65, 67, 68
Surface layer	Polysaccharide	*B. asaccharolyticus*	Precipitation, indirect hemagglutination	88
K	Protein (pili)	*B. nodosus*	Whole cell agglutination	18, 114, 120
O	LPS	*Bacteroides, Fusobacterium, Leptotrichia*	Indirect hemagglutination, precipitation	14, 17, 34, 36, 42, 46, 48, 51, 54–56, 58, 63, 66, 68, 70, 78, 80, 88, 90–92, 94, 121
P2	Protein (cell wall)	*F. nucleatum*	Precipitation, indirect hemagglutination, complement fixation	74–77
Toxic protein	Protein (cytoplasm)	*F. necrophorum*	Precipitation	35

rods with a similar trilaminar structure, and long ribbons (36, 57). Compared with the LPS isolated from *Fusobacterium*, suspensions of *B. fragilis* LPS contained relatively few structured particles, which often were difficult to visualize.

Chemical composition. The *Fusobacterium* LPS are complexes of polysaccharide and lipid, containing phosphorus and variable, but small, amounts of polypeptide (36, 78). All LPS isolated to date from *Fusobacterium* species contain glucosamine, heptose, and 2-keto-3-deoxyoctonate (34, 36, 48, 69, 78, 90). Glucose has been detected in nearly all LPS, and galactose and rhamnose have been detected in some (Table 2). The heptoses in the LPS examined in our laboratory were L-*glycero*-D-*manno*-heptose in all preparations and, in addition, D-*glycero*-D-*manno*-heptose in some LPS.

The chemical structure of the lipid component (lipid A) of *F. nucleatum* LPS was examined by Hase et al. (43). The backbone of lipid A consisted of a phosphorylated D-glucosamine disaccharide, viz., the type of backbone structure present in *Salmonella* LPS and in LPS from most other bacterial species (44). The fatty acids found were tetradecanoic, hexadecanoic, 3-hydroxytetradecanoic, and 3-hydroxyhexadecanoic acids.

The polysaccharide part of the *F. nucleatum* LPS macromolecule can be separated from lipid A by hydrolysis with 1% acetic acid. Recent fractionation studies on the polysaccharide part of LPS from one strain have indicated a polysaccharide core structure of 2-keto-3-deoxyoctonate, L-*glycero*-D-*manno*-heptose, glucose, glucosamine, and phosphorus (53). To this core is attached an oligosaccharide containing L-*glycero*-D-*manno*-heptose as a main component and, in addition, glucose, glucosamine, and an unknown amino compound. Different polysaccharide core structures may be present in LPS from other *Fusobacterium* strains.

These studies have definitely shown that *Fusobacterium* species have a typical LPS as part of their cell walls. An unusual feature is the presence of heptose in the O-specific oligosaccharide. Also, the amount of 2-keto-3-deoxyoctonate is unusually low (36, 53, 78).

As can be expected from the structure of lipid A, the *Fusobacterium* LPS are potent endotoxins (36, 116, 117).

The chemical composition of the LPS of *L. buccalis* is virtually unknown. Heptose and 2-keto-3-deoxyoctonate are present (48, 70), and the LPS is a powerful endotoxin (41). Possibly, the *L. buccalis* LPS has a chemical structure similar to that of LPS isolated from *Fusobacterium* species.

Preparations of LPS isolated from *Bacteroides* species by ultracentrifugation of the water phase after extraction of whole bacterial cells with phenol-water contained high amounts of carbohydrate, phosphorus, small amounts of lipid, and some polypeptide (14, 46, 50, 54, 91, 92). A substantial part of the carbohydrate may originate from glucans. Several sugar components have been found, but not heptose and 2-keto-3-deoxyoctonate. Recent studies have indicated that the *Bacteroides* LPS isolated by us are heterogeneous preparations.

The LPS isolated from *B. fragilis* by Kasper and Seiler (68) and from *B. asaccharolyticus* by Mansheim and Kasper (88) apparently are homogeneous products. Chemical examinations showed that heptose and 2-keto-3-deoxyoctonate were absent in both (66, 89). 3-Hydroxyte-

TABLE 2. *Sugars present in Fusobacterium LPS*[a]

Sugar	Presence in LPS[b] from:		
	F. nucleatum (20 strains)	*F. necrophorum* (4 strains)	Other species[c]
Glucosamine	++	++	++
2-Keto-3-deoxyoctonate	++	++	++
L-*glycero*-D-*manno*-Heptose	++	++	++
D-*glycero*-D-*manno*-Heptose	+	+	+
Glucose	++	+	+
Galactose	+	+	+
Rhamnose	+	−	+

[a] Data from reference 34 and unpublished observations.

[b] ++, Present in all LPS; +, present in some LPS; −, not detected.

[c] Including 1 to 3 strains of *F. varium, F. russii, F. mortiferum, F. gonidiaformans.*

tradecanoic acid, a regular component of other LPS, was not found in the *B. asaccharolyticus* LPS.

It emerges from these studies that the chemical structure of the LPS of *Bacteroides* species is in fact virtually unknown. The few pertinent findings are the absence of heptose, 2-keto-3-deoxyoctonate, and, possibly, 3-hydroxytetradecanoic acid, indicating the presence in *Bacteroides* of an atypical LPS. This indication is supported by the finding of a weak endotoxic activity (66, 89, 116, 117).

Immunological properties. The serological activity of LPS isolated from *Bacteroidaceae* has been examined by indirect hemagglutination and precipitation techniques, using antisera produced in rabbits by immunization with whole microbial cells. Antisera to whole cells have been used because rabbit antisera to purified LPS commonly are low titer (47; unpublished observations). To get maximal sensitization of the sheep erythrocytes used in indirect hemagglutination, LPS has to be treated with diluted NaOH.

de Araujo et al. (17), using hemagglutination inhibition, found a high degree of serological specificity of LPS isolated from 21 strains of *F. nucleatum*. The findings were corroborated by Kristoffersen et al. (80), who detected four antigenic specificities in LPS prepared from four *F. nucleatum* strains. One antigenic determinant was common to two of the LPS examined, and another was shared by LPS from three strains. Immunoprecipitates obtained by mixing LPS containing two different determinants with antibodies specific for one of the determinants harbored both. All serological activity was destroyed by oxidation with periodate. The results of these studies are clear indications of the presence of O-antigenic specificity in *F. nucleatum*.

By double diffusion in agar, purified LPS from *F. nucleatum* produced two lines of precipitation against homologous antiserum (72, 73).

Type specificity is also present in *F. necrophorum* LPS (90). LPS isolated from four strains of *B. melaninogenicus* contained shared and type-specific antigenic determinants (47). Using hemagglutination inhibition, Hofstad (50) detected six antigenic specificities in LPS isolated from three strains of *B. fragilis*. Each LPS had its own specificity and antigenic determinants shared with LPS from each of the other two strains. When LPS from two other *B. fragilis* strains were included, the number of antigenic specificities within the three LPS preparations increased to nine (52). Some antigenic specificities were shared by a few LPS prepared from strains belonging to other *Bacteroides* species. All serological activity disappeared after treatment of the LPS preparations with periodate. Multiple lines were obtained when LPS extracted with phenol-water from strains belonging to *B. fragilis* and other species within the *B. fragilis* group were examined by double diffusion in agar and immunoelectrophoresis (92).

Because of the chemical and physical heterogeneity of LPS prepared from *Bacteroides*, the results of the serological studies are not easily interpreted. A pronounced multispecificity seems to be present. The antigenic determinants disclosed by the hemagglutination inhibition experiments are most likely oligosaccharides. As long as the antigens are part of the cell wall polysaccharide–lipid–protein complex, the designation O antigens is justified. Some of the serological activity may, however, originate from other high-molecular-weight polysaccharides extracted together with the LPS.

Capsular or Surface Layer Antigens

Encapsulation in *B. fragilis* was occasionally observed by earlier workers. This finding has been corroborated by Kasper and collaborators (65, 67) and Babb and Cummins (3). There is some disagreement concerning the definition of the capsule in *B. fragilis*. Kasper (65) defined the capsule as an antigenic high-molecular-weight (about 0.2 μm thick) polysaccharide material external to the multilayered cell wall which could be made visible in the electron microscope by staining with ruthenium red. This external polysaccharide antigen was found to be species specific for *B. fragilis* (67). Babb and

Cummins (3) found encapsulated cells in cultures of *B. fragilis*, *B. thetaiotaomicron* (*B. fragilis* subsp. *thetaiotaomicron*), *B. vulgatus* (*B. fragilis* subsp. *vulgatus*), and *B. ovatus* (*B. fragilis* subsp. *ovatus*). The capsule had a thickness of up to four times the cell diameter. As suggested by Babb and Cummins, the polysaccharide of Kasper may represent a cell surface antigen rather than a true capsule. The use of sodium deoxycholate to separate the antigen from other membrane components (65, 68) supports this suggestion.

Kasper and Seiler (68) isolated the outer membrane complex of *B. fragilis* by gentle treatment of whole cells with ethylenediaminetetraacetate. The cell surface polysaccharide antigen was separated from the other membrane components by chromatography and treatment with sodium deoxycholate and trypsin (65, 68). The antigen preparation contained approximately 50% hexose, 25% hexosamine, and 5% methylpentose (65). The antigen was immunogenic in rabbits when mixed with bovine serum albumin. The antibodies were detected in a radioactive antigen-binding assay (65) and in indirect immunofluorescence tests (67). It is of particular interest that pelvic implants in rats of the isolated antigen mixed with sterile fecal contents produced abscesses (97). Also, the antigen may play a role for in vivo adherence of *B. fragilis* (98).

A similar surface antigen was isolated from two encapsulated strains of *B. asaccharolyticus* (88). The isolated material contained mainly galactose and, in addition, glucose and glucosamine. By double diffusion in agar, a single line of precipitation was produced against antiserum to either of the two strains.

In 1971 Takazoe et al. (119) isolated a capsular antigen from a saccharolytic strain of *B. melaninogenicus* by extraction of whole cells with acetate buffer. The antigen sensitized sheep erythrocytes to agglutination in rabbit antiserum prepared against whole bacterial cells. When examined by slide agglutination in a specific-factor antiserum, 68 of 219 strains of *B. melaninogenicus* isolated from the gingival crevices of human adults gave positive reactions (118). Strains possessing the capsular antigen were particularly virulent in an experimental guinea pig model.

P2 Antigen of *F. nucleatum*

In 1969 Kristoffersen (74, 75) isolated a group-reactive protein antigen, designated P2, from a strain of *F. nucleatum*. The purification procedure included extraction of crushed, dried, and defatted microbial cells with phosphate buffer, fractional precipitation with ammonium sulfate, ultracentrifugation, and different forms of chromatography. The purified preparation was immunogenic in rabbits (76). By double diffusion in agar or agarose the antigen gave a single line of precipitation with homologous or heterologous antiserum. Identical precipitation lines were produced by all of 29 strains of *F. nucleatum* (77). The antigen reacted in complement fixation tests and sensitized sheep cells to agglutination in antisera made against the homologous and heterologous *F. nucleatum* strains. The antigen was not found in *L. buccalis* strains.

Other Antigens

An intracellular toxic protein was partly purified from a bovine strain of *F. necrophorum* by Garcia et al. (35). Microbial cells were disintegrated by ultrasonication, and the toxin was precipitated from the cytoplasmic fraction by ammonium sulfate. The heat-labile toxic component was non-dialyzable and was sedimented by ultracentrifugation at 105,000 × *g*, indicating that the toxin itself might be either a high-molecular-weight protein or bound to a high-molecular-weight, nontoxic protein. The preparation reacted with homologous rabbit antiserum by precipitation.

Egerton (18) reported the presence in *B. nodosus* of somatic O antigens and a heat-labile cell surface antigen referred to as K antigen. In the presence of antiserum, cells with K antigen exhibited a distinctive agglutination characterized by flocculent granules. Forty-six strains of *B. nodosus* were divided into three serological types according to their K antigens. The antigen could be removed by washing the bacteria in buffered saline or water. Electron microscopic studies revealed that it was associated with the pili (114, 120).

IMMUNE RESPONSES TO *BACTEROIDACEAE*

The immune reaction to facultative organisms in health and disease have been extensively studied. Remarkably few studies have dealt with the immune response to anaerobic organisms.

Presence in Normal Human Subjects and in Animals of Antibodies to *Bacteroidaceae*

The demonstration of antibodies in normal human serum to members of the *Bacteroidaceae* goes back to 1936, when Feldman et al. (30) reported the occurrence in 2 of 14 healthy adults of agglutinins to *F. necrophorum*. Similar findings were reported by Danielsson et al. in 1974 (16).

Evans et al. (27) reported the presence in normal human serum of bactericidal antibodies to a strain of *F. nucleatum*. The antibodies were high-molecular-weight antibodies sensitive to 2-mercaptoethanol. Using crude extracts of one strain each of *B. fragilis*, *B. vulgatus*, *F. nucleatum*, and *F. mortiferum* as antigens in the indirect hemagglutination test, Quick et al. (101) found antibodies in a titer of 4 or more in a majority of normal human adults. Results of 2-mercaptoethanol treatment and comparison of titers of a few sera from mother-umbilical cord pairs indicated that the antibodies were primarily of the IgM class of immunoglobulins but that IgG antibodies might be present in a few sera.

Characteristically, the antibodies described above were IgM antibodies present in small amounts, making the use of sensitive serological techniques necessary for their detection. A substantial part of the antibodies found in human serum in the absence of infection or overt antigenic stimulation are directed against microbial polysaccharides. This seems to be true for normally occurring antibodies with specificites to members of the *Bacteroidaceae*. The presence of antibodies in normal human serum reacting with a crude LPS from *L. buccalis* was reported in 1965 by Mergenhagen et al. (93). The observation was later corroborated by Falkner and Hawley (29) and Hawley and Falkner (45).

Courant and Gibbons (11) found antibodies agglutinating sheep erythrocytes sensitized with a mixture of LPS isolated from eight strains of *Bacteroides* forming black-pigmented colonies on blood agar plates (*B. melaninogenicus* and/or *B. asaccharolyticus*) in each of 16 human serum samples. Antibodies were also detected in samples of serum using erythrocytes sensitized with LPS from single strains of black-pigmented *Bacteroides* isolated from the respective serum donors. Hofstad (49) examined serum samples from healthy children and blood donors for antibodies reacting with sheep erythrocytes sensitized with LPS purified from *B. melaninogenicus*, *B. fragilis*, and *F. nucleatum* (Table 3). Among the children there was an increasing frequency of positive reactions with increasing age. The antibodies detected in the sera of blood donors were all sensitive to 2-mercaptoethanol. Absorption experiments indicated that they were specific for each of the bacterial species tested and were directed against the polysaccharide moiety of the LPS.

Normal human serum contains antibodies reacting with the P2 antigen of *F. nucleatum* (77, 79). These antibodies were demonstrated by indirect hemagglutination in 86 of 99 blood donor sera in titers up to 4,096, as well as in 15 of 21 umbilical cord sera. A few high-titer sera and commercial human gamma globulin gave a precipitation line with the antigen when examined by double diffusion in agar. The antibodies, which were resistant to treatment with 2-mercaptoethanol, were eluted from Sephadex G-200 columns with the IgG class.

TABLE 3. *Frequency of positive indirect hemagglutination titers (≥10) of cord sera and sera from healthy children and blood donors to LPS isolated from B. fragilis, B. melaninogenicus, and F. nucleatum*[a]

Serum source	No. positive[b] with LPS from:		
	B. fragilis	*B. melaninogenicus*	*F. nucleatum*
Umbilical cord	0/10	0/10	0/10
Healthy children	5/12	0/12	1/12
<1 year old	19/20	12/17	11/20
1–4 years old	24/24	20/24	21/24
5–14 years old	51/54	43/54	54/54
Blood donors			

[a] Modified from reference 49.

[b] Number of sera with positive reactions/number of sera tested.

The presence in normal human subjects of secretory IgA antibodies to *Bacteroidaceae* has not been examined. Ste-Marie et al. (105) found IgA antibodies to *B. fragilis* in breast fluids of lactating women. Although the amount of IgA was 23- to 30-fold higher in breast fluid than in serum, the concentration of specific IgA antibodies was the same in both fluids, indicating that the antibodies were derived from serum.

More than 40 years ago Feldman et al. (30) carried out a comprehensive study of the occurrence of antibodies to *F. necrophorum* in the serum of different animals. Agglutinating antibodies were regularly present in cattle, sheep, swine, and horses. The titers varied from 25 to 6,400 and were commonly higher in adult cattle, ewes, and old swine than in calves, lambs, and young swine. A few sera from dogs kept in cages under laboratory conditions, from white rats, and from rabbits also gave positive reactions, but the titers were low.

Bactericidal and agglutinating antibodies to *B. nodosus* have been found in the sera of normal sheep (20, 23). The agglutinins showed specificities to the O antigen and, less regularly, to the K antigen (21).

Antibodies in low titers to a mouse strain of *Bacteroides* were detected in normal mouse serum (32). Mice were unresponsive to antigenic challenge with the *Bacteroides* strain, which cross-reacted with homogenates of intestinal tissues of neonatal mice (33).

Normal rabbit serum often contains antibodies agglutinating sheep erythrocytes sensitized with *L. buccalis* LPS (29).

The results of the above-mentioned studies indicate that humans and animals are exposed to continuous stimulation from antigens derived from members of the *Bacteroidaceae* normally present on the mucous membranes. That some antibody formation is induced by cross-reacting microbial antigens cannot be excluded, however.

Production of Antibodies in Response to Infections with Members of the *Bacteroidaceae*

The first report of antibody production in response to anaerobic infection in humans is that of Johan (64), who found complement-binding antibodies in sera of patients suffering from severe suppurative infections caused by *F. necrophorum*. Antibodies to *F. necrophorum* were also found in patients with *F. necrophorum* septicemia (6, 86) and in serum of patients with ulcerative colitis or other ulcerative lesions of the bowel (12, 13).

In more recent years the production of humoral antibodies to infecting anaerobic organisms has been examined by some authors. Caselitz et al. (8) reported the appearance of specific agglutinins and complement-fixing antibodies in the sera from three patients with *F. necrophorum* septicemia. In a patient with a perirectal abscess from which *B. fragilis* and *Clostridium difficile* were isolated, Danielsson et al. (15) found antibodies to the homologous *B. fragilis* strain detectable with bacterial agglutination, double diffusion in agar, and indirect immunofluorescence. In later studies (16, 82) an antibody response was demonstrated in patients with *Fusobacterium* and *Bacteroides* bacteremia and in patients with suppurative lesions associated with *B. fragilis*. The occurrence in sera from patients with *B. fragilis* infections of precipitating antibodies reacting with sonic extracts of laboratory *B. fragilis* strains was reported by Rissing et al. (103). Using indirect hemagglutination and the indirect fluorescent antibody technique, Schwan and Rydén (106) observed significant titer changes in IgG and IgA antibodies, but less consistently in IgM antibodies, during the disease and convalescent periods of patients with *B. fragilis* infection. The rise in antibody titers seemed to take place within 1 week after the onset of the disease, suggesting the involvement in the IgG response of a booster effect. The titers decreased slowly over the following 3 to 12 months.

It is clear from these studies—in particular, those of Danielsson and co-workers (15, 16, 82) and Schwan and Rydén (106)—that anaerobic infections in humans involving members of the *Bacteroidaceae* are associated with the production of specific antibodies. Whereas the normally occurring serum antibodies are mainly IgM antibodies, antibodies of the IgG class seem to be involved in the immune response associated with infection. This may be of value in an eventual future evaluation of serodiagnosis as a tool in the diagnosis of *Bacteroides* infections. A prerequisite for this is a better knowledge of the antigenic makeup of the *Bacteroidaceae*. Especially, the presence in this group of microorganisms of group-reactive or species-specific antigens should be studied.

Concerning anaerobic infections in animals, a rise in antibody titers to *B. nodosus* has been observed in sheep suffering from foot rot (21).

Cell-Mediated Immunity and Local Immune Responses to *Bacteroidaceae*

Cell-mediated immunity to anaerobic bacteria has been studied to some extent in human subjects with periodontal disease. Ivanyi and Lehner (60) found that sonic extracts of some microorganisms present in dental plaque, among them *B. melaninogenicus* and *F. nucleatum*, stimulated lymphocyte transformation in individuals with gingivitis and with mild or moderate periodontitis. Subjects with severe periodontitis had significantly reduced responses, thought to result from blocking serum factors (62). There was a correlation between the responses of lymphocytes to bacterial sonic extracts and to sonic extracts of autologous dental plaque (61). Patters et al. (99) studied the blastogenic response of lymphocytes to supernatants of soluble sonic extracts of different plaque organisms in individuals with various severities of periodontal disease and in normal and edentulous subjects. The lymphocyte response of subjects with periodontitis to *B. melaninogenicus* and *L. buccalis* antigens was significantly greater than that of normal and edentulous subjects. Similar findings were reported by Budtz-Jørgensen et al. (7), whereas Smith et al. (111) found unsignificant blastogenic responses to sonic extracts of *F. nucleatum* and *B. melaninogenicus* in human adults during the onset and recovery from experimental gingivitis (87). Immediate hypersensitivity reactions have been described in individuals with periodontitis skin-tested with extracts of *F. nucleatum* and *B. melaninogenicus* (96).

Gingivitis and periodontitis possibly are complex multifactorial diseases. The histopathology of chronic periodontal disease is consistent with an immune response to a continuous antigenic stimulation. Lymphocytes and, especially in the

advanced stages, plasma cells are the dominating cell types of the gingival tissue. Soluble antigens from plaque material have been demonstrated in the gingival tissues (102). Bacterial antigens (10) and specific antibodies (5) have been detected. Obviously, immune reactions may take place in the inflamed tissue. The nature of these reactions and the degree to which antigens from *Bacteroides*, *Fusobacterium*, and *Leptotrichia* are involved have not been explored.

The histological picture of a suppurative anaerobic infection is that of an acute infection. The immune reactions taking place in such infections are virtually unknown. Monteiro et al. (95) have reported the presence in rectal and colonic mucosa in ulcerative colitis of antibodies reacting with gram-negative anaerobic rods, possibly *Bacteroides*.

ATTEMPTED SEROLOGICAL CLASSIFICATION OF *BACTEROIDACEAE*

Up to recent years the anaerobic, nonspore-forming, gram-negative rods formed a taxonomically ill defined group of microorganisms. Several serological studies were performed to clarify taxonomic relationships. These studies have been reviewed by Werner (122) and Sonnenwirth (112). The studies, which were carried out by conventional agglutination, precipitation, complement fixation, and hemagglutination techniques, showed no serological relationship between *Bacteroides* and *Fusobacterium*. Also, there was a lack of cross-reactions between the *B. fragilis* group and *B. melaninogenicus*. Some cross-reactivity was observed between species of the *B. fragilis* group. The cross-reactions were generally weak as opposed to the stronger reactions in homologous antigen-antibody systems, indicating the presence of strain- or type-specific antigens or antigens which might be species specific.

The serological relationships between species within the *B. fragilis* group were particularly studied by Beerens and co-workers (4, 108). Based on a high agglutination titer in one of six antisera prepared against selected type strains, they were able to classify 131 clinical isolates into seven serotypes: A, B, C, D, E_1, E_2, and E_1E_2. A number of minor cross-reactions permitted a further subtyping. The serotypes corresponded fairly well to *B. thetaiotaomicron* (A), *B. ovatus* (B), *B. vulgatus* (C), *B. distasonis* (*B. fragilis* subsp. *distasonis*) (D), and *B. fragilis* (E_1, E_2, or E_1E_2). The serotypes E_1, E_2, and E_1E_2 presented a high degree of serological heterogeneity. The heterogeneity of *B. fragilis* is evident also from biochemical studies (59) and from serological studies carried out by Lambe and Moroz (84). By agglutination of whole microbial cells in absorbed antisera, 45 serological patterns were recorded in 98 *B. fragilis* strains isolated from clinical specimens and normal human feces. Elhag and co-workers (25, 26), using agglutination of heated cells in nonabsorbed and absorbed antisera, recorded 19 serotypes among 20 selected strains of *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*.

The antigens reacting by agglutination in nonabsorbed or absorbed sera most likely are polysaccharide haptens. Such antigens are seldom species specific. Another approach would be to search for major protein antigens, which sometimes are more characteristic for a genus or a species than are polysaccharide haptens. Kasper and Seiler (68) thus found that the outer membrane peptide patterns of five strains of *B. fragilis*, as judged from sodium dodecyl sulfate-polyacrylamide electrophoresis, were remarkably similar to one another and different from those of the related species.

The best approach to a serological classification of the species within the *B. fragilis* group has been made by immunofluorescence techniques. Griffin (40) prepared conjugates against strains of *B. fragilis* which seemed to be species specific. Relatively few strains were examined. Abshire et al. (2) produced antisera to 7 strains of *B. fragilis* and used direct and indirect fluorescent antibody techniques for serological identification of 24 *B. fragilis* strains classified as such by conventional bacteriological methods. They observed subspecies, and also strain, specificities with a few sera. Their conclusion was that the indirect fluorescent antibody technique was excellent for identification of *B. fragilis* but that a number of antibody pools encompassing a wide antigenic coverage had to be worked out. Promising results were also reached by Kasper et al. (67), using indirect immunofluorescence and antisera with specificity for the cell surface polysaccharide (capsular) antigen.

In 1954 Pulverer (100) reported that *B. melaninogenicus* strains could be classified into 4 serotypes. Later, 11 serotypes were described (71). Several *B. melaninogenicus* serotypes were also reported by Werner and Sebald (124). Lambe (81) prepared fluorescent antibody reagents that were specific for *B. asaccharolyticus*, *B. melaninogenicus* subsp. *melaninogenicus*, and *B. melaninogenicus* subsp. *intermedius*. An additional *B. melaninogenicus* subsp. *intermedius* serogroup was described later (83).

Sharpe (107) studied the serological relationships between 26 rumen strains of *B. ruminicola*, *B. amylophilus*, and *B. succinogenes*. She used

precipitation, agglutination, and immunofluorescence techniques and found that the strains could be divided serologically into three groups broadly corresponding to the species as defined by their physiological characteristics. There was some cross-reactivity between *B. ruminicola* and a few human strains of *B. oralis* and *B. melaninogenicus*.

The serological relationships between *Fusobacterium* species have not been examined systematically. Werner (123) found cross-reactivity between *F. varium* (*Sphaerophorus varius*) and *F. mortiferum* (*S. freundi*) dependent on the presence of heat-labile antigens. O-antigenic specificity may form a basis for serotyping *F. nucleatum* (80).

Although a useful tool for the classification of organisms within the *Bacteroidaceae*, serology is no substitute for conventional bacteriological methods, including gas-liquid chromatography of end products from glucose (or peptone) fermentation. Serological methods may be used for the typing of isolates within a species. However, infections caused by *Bacteroides* or *Fusobacterium* species are all endogenous, which limits the practical value of serotyping.

DETECTION BY IMMUNOFLUORESCENCE OF *BACTEROIDACEAE* IN CLINICAL SPECIMENS

The usability of fluorescent antibody procedures as compared with anaerobic cultivation for identification of *Bacteroidaceae* in direct smears of clinical materials was examined by Garcia et al. (39), Fales and Teresa (28), and Stauffer et al. (113). Garcia et al. prepared a fluorescent antibody conjugate specific to a bovine strain of *F. necrophorum* and used the conjugate for detection of *F. necrophorum* in liver abscesses, viscera, and ruminal contents of cattle. Fales and Teresa prepared monovalent and polyvalent conjugates for the detection of the same species in materials from bovine liver abscesses. Because of some serological heterogeneity between the test strains, a pool of selected monovalent conjugates was most reliable. Weak cross-reactions with some other bacterial species presented no problems.

Stauffer and co-workers, using the indirect technique, prepared antisera against 31 strains belonging to different *Bacteroides* and *Fusobacterium* species. The antisera were either strain specific, specific for groups of strains within a species, or, occasionally, specific for strains representing different species. Thirty clinical specimens were tested. With a few exceptions, complete species agreement between direct smear immunofluorescence and culture results were obtained by using polyvalent antiserum pools.

These studies demonstrate the value of immunofluorescence for rapid presumptive identification of *Bacteroidaceae* in clinical specimens. Of particular interest is the finding of Kasper et al. (67) that 83 strains of *B. fragilis* all reacted positively in an indirect fluorescent antibody procedure, using an antiserum with specificity for the surface layer polysaccharide antigen. Only 1 of 32 strains of other species within the *B. fragilis* group gave a positive reaction.

IMMUNOPROPHYLAXIS OF INFECTIONS DUE TO MEMBERS OF THE *BACTEROIDACEAE*

Vaccination is effective in the protection of sheep against foot rot, which is a bacterial dermatitis of ungulates characterized by separation of the hoof. Foot rot in sheep is caused by *B. nodosus* in combination with *F. necrophorum*. *B. nodosus* is the only species that is able to transfer the disease from one animal to another. Egerton and Roberts (24) showed that intramuscular injections of Formalin-killed suspensions of *B. nodosus* or mixtures of *B. nodosus* and *F. necrophorum* protected sheep in pens against artificially induced foot rot, whereas Formalin-killed suspensions of *F. necrophorum* alone did not. Also, the vaccination seemed to have a therapeutic effect in sheep already infected. Their findings were corroborated by a series of subsequent studies (19, 22, 23). An extensive field study was performed by Skerman and Cairney (110). They prepared large batches of oil adjuvant vaccines from fluid cultures of five local strains of *B. nodosus* and examined the efficacies of the different vaccines on eight New Zealand farms, where a total of 1,500 trial sheep of various breeds were exposed to natural infection. The vaccines had a clear protective effect against foot rot in sheep on all farms. The effect lasted at least for 6 months. In addition, the vaccines showed a significant curative effect on established cases of foot rot. The nature of the protective immunogen(s) in the *B. nodosus* vaccines has not been clarified. There is some evidence that the protection is associated with antibodies to a heat-labile, high-molecular-weight surface antigen (115).

Liver abscesses in feedlot cattle caused by *F. necrophorum* (hepatic necrobacillosis) represent an economically important disease in meat-producing countries. Development of an effective vaccine is therefore highly desirable. In 1970, Roberts (104) reported that immunization of rabbits with Formalin-killed *F. necrophorum* in Freund adjuvant resulted in a low-grade immu-

nity to experimental *F. necrophorum* infections. A few years later, Garcia et al. (37) showed that an alum-precipitated toxoid from the cytoplasmic fraction of ultrasonically disrupted cells of *F. necrophorum* was able to reduce the incidence of bovine hepatic abscesses. Also, the alum-precipitated toxoid protected mice from a lethal challenge with *F. necrophorum* (38). Abe et al. (1) found that intraperitoneal injections in mice of suspensions in saline of *F. necrophorum* with or without added aluminium hydroxide adjuvant gave a protection of up to 77.5% against experimental infections with *F. necrophorum*. Attempts at oral vaccination only resulted in a delay in time of death. The results of these studies indicate that a future vaccination against necrobacillosis is possible.

For the time being, vaccination of human subjects against infections caused by *Bacteroides* or *Fusobacterium* is not being considered. Anaerobic infections can be adequately treated with antibiotics alone or in combination with surgery. The problems which may arise are mainly associated with an early clinical and bacteriological diagnosis.

CONCLUDING REMARKS

The importance of the *Bacteroidaceae* in health and disease has been adequately documented through the last decades. What remains to be studied in more detail is the relationship between the microorganisms and the host. Immunology plays a significant role in this relationship.

The present review has mainly been concerned with antigens isolated from *Bacteroidaceae* and the antibody response in humans and animals to these antigens and to whole microbial cells. The presence in *Bacteroidaceae* of cell wall LPS and other cell envelope antigens has been demonstrated, the presence of specific antibodies has also been demonstrated in healthy individuals. A few studies have clearly indicated that infections in humans involving *B. fragilis* and other anaerobic microorganisms are accompanied by production of antibodies to the infecting bacterial strains. Serology has proved useful in the taxonomy of *Bacteroidaceae* and, more important, in the laboratory diagnosis of *B. fragilis* infections.

The antigens isolated and purified from members of the *Bacteroidaceae* are, however, few in number. The study of the immune response is still in its beginning. More work has to be done regarding immunological methods for detection and identification of the pathogenic species. The *Bacteroidaceae* represent a challenge not only to bacteriologists and clinicians but also to immunologists and biochemists.

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