DEGRADATION OF HUMAN IMMUNOGLOBULINS G AND M AND COMPLEMENT FACTORS C3 AND C5 BY BLACK-PIGMENTED *BACTEROIDES*

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SUMMARY. Strains of *Bacteroides*, *Capnocytophaga* and *Fusobacterium* were examined by immunological methods for their ability to degrade the human serum proteins IgG, IgM, C3 and C5. The proteolytic activity of the strains was measured in terms of the breakdown of serum into trichloroacetic acid-soluble material. Only black-pigmented *Bacteroides* strains showed proteolytic activity. Strains of *B. gingivalis* degraded IgG, IgM, C3 and C5, strains of *B. intermedius* IgG and C3, strains of *B. endodontalis* C3 and IgG and a strain of *B. loeschei* degraded only IgG. These findings are discussed in relation to the pathogenicity of the black-pigmented *Bacteroides*.

INTRODUCTION

Some clinically important bacteria produce enzymes that are able to cleave plasma proteins involved in the host defence against microbial infections. Immunoglobulin A (IgA) proteases are produced by *Neisseria gonorrhoea*, *N. meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. These bacteria colonise the IgA-coated mucous membranes and are all pathogenic to man (Plaut *et al.*, 1975; Kilian, Mestecky and Schrohenloher, 1979; Male, 1979). IgA is also degraded by strains of *Bacteroides gingivalis*, *B. intermedius* and *B. melaninogenicus* (Werner and Müller, 1971; Kilian, 1981) which are suspected to be pathogens in periodontal disease. They have also been isolated from abscesses adjacent to the oral cavity. Some of these *Bacteroides* strains may also produce IgG-cleaving enzymes (Werner and Müller, 1971; Kilian, 1981).

Bacteria may release substances which cause consumption of serum complement. *Pseudomonas aeruginosa*, *Serratia marcescens* and β -haemolytic streptococci all produce proteinases that are able to cleave human serum complement components C3 and C5 (Ward *et al.*, 1973; Schultz and Miller, 1974). We have recently reported that C3 is extensively degraded by *B. gingivalis* *in vitro* and in experimentally infected guinea pigs (Sundqvist *et al.*, 1984).

In the present study, the ability of strains of the genera *Bacteroides*, *Fusobacterium* and *Capnocytophaga* to degrade IgG, IgM, C3 and C5 in human serum was assayed. Special precautions were taken to exclude the possibility that C3 was eliminated by activation of serum complement by the bacteria.

MATERIAL AND METHODS

Bacterial strains and culture conditions. The strains tested are listed in table I. Reference strains were obtained from the American Type Culture Collection (ATCC), Rockville, MD; the National Collection of Type Cultures (NCTC), Colindale, UK; Virginia Polytechnic Institute (VPI), Blacksburg, VI; and the National Collection of Dairy Organisms (NCDO), Reading, UK. *B. asaccharolyticus* strain B537 and *B. gingivalis* strains W83, W50, 381, 376 were obtained from T.J.M. van Steenberg, Department of Oral Microbiology, Vrije Universiteit, Amsterdam, The Netherlands. Other strains were isolated by the authors from dental root canals. All the strains were stored on blood agar in an anaerobic box with an atmosphere of CO₂ 5%, H₂ 10% and N₂ 85%. The blood-agar medium contained defibrinated horse blood haemolysed by freezing and thawing (Holdeman, Cato and Moore, 1977). To suspend bacteria a previously described medium (Neidhardt, Bloch and Smith, 1974) was modified to contain 40 mM 3-N-morpholino-propane-sulphonic acid, 4 mM tricine (Sigma Chemical Co., St Louis, MO, USA), 0.3 mM potassium sulphate, 0.5 µM calcium chloride, 0.5 mM magnesium chloride, and 50 mM sodium chloride. It was adjusted to pH 7.4 with potassium hydroxide, sterilised by filtration and designated MOPS.

Assay of proteolytic activity. Bacteria harvested from blood-agar plates after incubation for 4 days were suspended in MOPS to a density of c. 10⁹ cfu (0.3 mg dry weight)/ml. The proteolytic activity of bacterial cells was assayed in anaerobic conditions in a reaction mixture containing 0.6 ml of pooled serum from healthy adult donors and 0.3 ml of bacterial suspension. After incubation at 37°C for 24 h, 0.1 ml of the reaction mixture was precipitated in 0.4 ml of ice-cold 0.25 M trichloroacetic acid. After centrifugation, the extinction of the supernatant fluid was read at 280 nm. The standard was 1 mM L-tyrosine in 0.25 M trichloroacetic acid.

Analysis of serum proteins. Samples of the reaction mixtures were centrifuged to remove bacteria. They were assayed for IgG and IgM by radial immunodiffusion and for C3 and C5 by rocket immunoelectrophoresis (Laurell, 1972). Immunodiffusion was performed in a humidified chamber at 23°C for 24 h. To samples to be analysed for C3 and C5, EDTA was added to a final concentration of 5 mM before electrophoresis. Samples (5 µl) were applied to wells (2.5 mm) in agarose gels (HSA; Litex, Glostrup, Denmark) (1% w/v) in Tris-barbital buffer (pH 8.6). Electrophoresis was performed at 10 V/cm for 4 h in the same buffer.

The electrophoretic homogeneity of C3 in the treated sera was studied by crossed immunoelectrophoresis (Ganrot, 1972). Samples (5 µl) were electrophoresed at 4 V/cm in agarose gel 1% w/v in Tris-barbital buffer (pH 8.6) until bromophenol blue-marked albumin had moved 40 mm in the gel. The second dimension run into the antibody-containing gel was performed at 2 V/cm for 20 h in the same buffer. Antisera against IgG, IgM, C3c, C3d, C5 (Dakopatts A/S, Copenhagen, Denmark) and C3 (β1c/β1A) (Cappel Laboratories, Cochranville, PA, USA) were used.

After electrophoresis, agarose gels were washed with saline (sodium chloride 0.85% w/v) to remove excess protein and were stained with Coomassie brilliant blue R-250, 0.1% w/v, (Serva Feinbiochemica, Heidelberg, West Germany) in ethanol 50% and acetic acid 10% in water. Gels were destained in ethanol 50% and acetic acid 10% in water.

Proteins of the bacteria-treated sera were also separated by polyacrylamide gradient gel electrophoresis (Pharmacia Fine Chemicals, Uppsala, Sweden). The gradient gel slabs contained 4–30% polyacrylamide, and electrophoresis was run in a Tris buffer (pH 8.4), containing 90 mM Tris, 80 mM boric acid and 2.5 mM EDTA. The samples were prepared by mixing 25 µl of the cell-free fluid, 10 µl of a 40% w/v sucrose solution, and 0.5 µl of a solution of bromophenol blue 1% in ethanol. A 3-µl sample of this mixture was applied to the gel and the electrophoresis was run at 4°C for 24 h at 120 V. After the run the proteins were fixed and stained with Coomassie brilliant blue R-250.

Removal of complement component C4 and Factor B from serum. The effect of the bacteria was also assayed on sera depleted of C4 and Factor B. Affinity chromatography was used to remove these factors as described by Löfgren, Tärnvik and Carlsson (1980). The absorbed serum was assayed for IgG, IgM, IgA, C3, C4 and Factor B by radial immunodiffusion in Partigen plates (Behringwerke AG, Marburg, West Germany). There was no detectable C4 and Factor B in the depleted serum. The levels of IgG, IgM, IgA and C3 were not affected by the chromatography.

There was no haemolytic activity by either the classical or alternative pathway in the C4- and Factor B-depleted serum.

Assay of complement activation. The activation of complement by the alternative pathway was determined by a technique based on haemolysis in gel of rabbit erythrocytes (Fong and Renaud, 1978) and the activation of complement by the classical pathway with antibody coated sheep erythrocytes (Lange, 1962).

RESULTS

Proteolytic activity of bacterial cells as measured by degradation of serum into trichloroacetic acid-soluble material within 24 h was demonstrated in four of the 29 tested species of the genera *Bacteroides*, *Fusobacterium* and *Capnocytophaga*. These were the black-pigmented *Bacteroides*: *B. gingivalis*, *B. intermedius*, *B. asaccharolyticus* and *B. endodontalis* (table). Of the 22 strains of these four species that were tested, only two strains of *B. asaccharolyticus* were not proteolytic. As demonstrated by polyacrylamide gradient gel electrophoresis, strains of *B. gingivalis* hydrolysed most of the serum proteins into fragments of mol. wt $< 30 \times 10^3$ (fig. 1). Strains of *B. intermedius* also degraded many of the serum proteins. The following strains showed no proteolytic activity and no degradation of C3, C5, IgG or IgM: *B. buccae* ATCC33574, *B. buccalis* NCDO2354, *B. coagulans* ATCC29798, *B. denticola* NCDO2352, *B. distasonis* ATCC8503, *B. fragilis* NCTC9343 and NCTC10584, *B. oralis* ATCC33269, *B. oris* ATCC33573, *B. ovatus* ATCC8483, *B. pentosaceus* NCDO2353, *B. ruminicola* ss.

FIG. 1.—Polyacrylamide gradient gel electrophoresis of normal human serum incubated for 24 h at 37°C with cells of *B. gingivalis* (W83, track 2; 381, track 3), *B. intermedius* (NCTC9336, track 4; UJB13-c, track 5), *B. asaccharolyticus* (NCTC9337, track 6), *B. endodontalis* (BN11a-f, track 7), *B. loeschei* (ATCC15930, track 8), *B. melaninogenicus* (ATCC25845, track 9) and *F. nucleatum* (NCTC10562, track 10). Human serum incubated without bacteria was in track 1.

TABLE

Proteolytic activity and degradation of complement components C3 and C5 and of IgG and IgM in human serum by strains of black-pigmented Bacteroides

Strain	Site of isolation	Proteo- lytic acti- vity*	Degradation† of			
			C3	C5	IgG	IgM
<i>B. asaccharolyticus</i>						
ATCC25260	Empyema	—	—	—	—	—
ATCC27067	Leg wound (man)	—	—	—	—	—
VPI4199	Faeces (man)	+	—	—	—	—
NCTC9337	Infected haemorrhoids	+	—	—	—	—
B537	Leg lesion	+	—	—	—	—
<i>B. endodontalis</i>						
H11a-e	Infected root canal	+	+	—	++	—
BN11a-f	Infected root canal	+	+	—	—	—
<i>B. gingivalis</i>						
W83	Clinical specimen	+	+++	+++	+++	+++
W50	Clinical specimen	+	++	+++	—	++
381	Subgingival plaque	+	+++	+++	+++	+++
BE-c	Infected root canal	+	++	+++	—	++
TN42-c	Infected root canal	+	+++	+	+++	+
376	Periodontitis	+	+++	+++	+++	+++
<i>B. intermedius</i>						
NCTC9336	Vincent's gingivitis	+	+	—	+++	++
AB13a-f	Infected root canal	+	+	—	+++	—
ABg-c	Infected root canal	+	+	—	++	—
BO-b	Infected root canal	+	+	—	+++	—
PW-c	Infected root canal	+	+	—	++	—
SL-d	Infected root canal	+	+	—	++	—
SN-c	Infected root canal	+	+	—	—	—
SUN-k	Infected root canal	+	+	—	+++	—
UJB13-c	Infected root canal	+	+	—	+++	—
<i>B. loeschei</i>						
ATCC15930	Gingival crevice	—	—	—	+++	—
<i>B. melaninogenicus</i>						
ATCC25845	Human sputum	—	—	—	—	—
VPI9343	Not known	—	—	—	—	—
VPI13020	Periodontitis	—	—	—	—	—

*Proteolytic activity was defined as the ability of cells (0.3 mg dry weight) in 0.3 ml of MOPS dilution solution and 0.6 ml of human serum to form trichloroacetic acid-soluble material within 24 h.

†Degradation was scored as +++ = no detectable amount remaining after 24 h; ++ = < 25%; + = ≥ 25% ≤ 75%; — = > 75% detectable after 24 h.

ruminicola ATCC19189, *B. thetaiotaomicron* NCTC10582, *B. ureolyticus* VPI7815 and VPI13309, *B. vulgatus* ATCC8482, *C. gingivalis* VPI12914, *C. ochraceus* ATCC27872, *C. sputigena* VPI13178, *F. gonidiaformans* ATCC25563, *F. naviforme* ATCC25832, *F. necrophorum* NCTC10575, *F. nucelatum* NCTC10562, *F. russi* ATCC25533, *F. varium* ATCC8501.

Only black-pigmented *Bacteroides* strains caused any decrease in the level of

immunoglobulins or complement components C3 and C5 as demonstrated by immunoelectrophoresis and immunodiffusion methods (table). Decreased levels of IgG were found in sera incubated for 24 h with four of the six strains of *B. gingivalis* and eight of the nine strains of *B. intermedius*. *B. loeschei* strain ATCC15930 and *B. endodontalis* strain H11a-e also caused a significant decrease of IgG. All strains of *B. gingivalis* tested and *B. intermedius* strain NCTC9336 caused a decrease in the level of IgM. *B. gingivalis* strains W83, 381 and 376 decomposed both IgM and IgG so efficiently that no traces of these proteins could be detected after 24 h. The amount of C3 was significantly decreased in sera treated with *B. gingivalis*, *B. intermedius* and *B. endodontalis* (table). No traces of C3 could be detected in sera treated with *B. gingivalis* strains W83, 381, 376 and TN42-c (fig 2) while strains W50 and BE-c had less effect on C3 (fig 2). *B. intermedius* strains and *B. endodontalis* strains caused a decrease of C3 although not as efficiently as *B. gingivalis* strains (fig 3). C5 could not be detected in sera treated with five of the *B. gingivalis* strains. None of the other species caused any significant decrease in C5 (table I).

The effect of some of the strains on C3 of serum samples was also evaluated by crossed immunoelectrophoresis. In the presence of *F. nucleatum* strain NCTC10562, C3 was converted into fragments as in serum incubated without bacteria (fig 4). In the

FIG. 2.—C3 assayed by rocket immunoelectrophoresis in serum incubated for 24 h at 37°C with *B. asaccharolyticus* strains ATCC25260, VPI4199, NCTC9337 and B537 (wells 2–5) and *B. gingivalis* strains W50, BE-c, 376, 381, W83 and TN42-c (Wells 6–11). Unincubated serum was in wells 1 and 12. Before electrophoresis, all sera were diluted with an equal volume of Tris-barbital buffer (pH 8.6) with 5mM EDTA.

FIG. 3.—C3 assayed by rocket immunoelectrophoresis in serum incubated for 24 h at 37°C with *B. loeschei* ATCC15930 (well 2), *B. melaninogenicus* ATCC25845 and VPI13020 (wells 3 and 4), *B. intermedius* NCTC9336, AB13a-f, ABg-c, BO-b, PW-c, SL-d, SN-c and UJB13-c (wells 5–12), *B. endodontalis* H11a-e (well 13), *C. gingivalis* VPI12914 (well 14), *C. sputigena* VPI13178 (well 15), *F. gonidiaformans* ATCC25563 (well 16), *F. nucleatum* NCTC10562 (well 17), and *F. russi* ATCC25533 (well 18). Unincubated serum was in wells 1 and 19. Before electrophoresis, all sera were diluted with an equal volume of Tris-barbital buffer (pH 8.6) with 5mM EDTA.

FIG. 4.—Crossed immunoelectrophoresis of normal human serum incubated for 24 h at 37°C without bacteria and with *F. nucleatum* NCTC10562. This *F. nucleatum* strain is representative of strains not causing any decrease in C3 in rocket immunoelectrophoresis. The cathode is to the left in the first dimension and at the bottom in the second dimension.

presence of *B. intermedius* strain NCTC9336 there was rapid conversion of C3 and the fragments were then further degraded as indicated by changes in their electrophoretic mobilities (fig 5). The most rapid conversion of C3 was induced by *B. gingivalis* strain W83. The fragments were further degraded and after 8 h only trace amounts could be detected (fig 6).

By depleting serum of Factor B and C4 both the spontaneous conversion of C3 and that induced by *F. nucleatum* were prevented. *B. intermedius* and *B. gingivalis* also degraded C3 in the depleted serum but at a slower rate and with an electrophoretic pattern different from that induced in normal serum (figs 5 and 6). After incubation for 24 h, no trace of C3 could be detected by immunoelectrophoresis with antisera against C3, C3c or C3d in serum incubated with *B. gingivalis* strains W83, 381, 376 or TN42-c.

In serum treated for 10 min with *B. gingivalis* strain W83 (fig 7), complement could not be activated by the classical or the alternative pathway. After 30 min, complement

FIG. 5.—Crossed immunoelectrophoresis of normal human serum (NHS) and serum depleted of C4 and Factor B incubated for 24 h at 37°C with *B. intermedius* NCTC9336. Electrode positions were as in fig. 4.

FIG. 6.—Crossed immunoelectrophoresis of normal human serum (NHS) and serum depleted of C4 and Factor B incubated for 24 h at 37°C with *B. gingivalis* W83. Electrode positions were as in fig. 4.

FIG. 7.—Effect of cells of *B. gingivalis* W83 (●), *B. intermedius* NCTC9336 (▲) and *F. nucleatum* NCTC10562 (■) on the complement activity of human serum. Bacteria were incubated in serum, and the percentages of complement activity by the classical (A) and the alternative (B) pathways remaining after various periods of time were recorded as means of six experiments.

in serum treated with *B. intermedius* strain NCTC9336 could not be activated by the alternative way, whereas serum treated with *F. nucleatum* strain WCTC10562 contained complement which could be activated by both pathways even after exposure for 60 min to the bacterial cells.

DISCUSSION

Several authors have reported that strains of black-pigmented *Bacteroides* inhibit the phagocytic killing of other bacteria present in in-vitro systems (Ingham *et al.*, 1977; Tofte *et al.*, 1980; Ingham *et al.*, 1981; Jones and Gemmell, 1982). Also, in experimental polymicrobial infections in guinea pigs, the addition of strains of black-pigmented *Bacteroides* to an avirulent mixture of bacteria can promote the survival of those bacteria despite the presence of large numbers polymorphonuclear leukocytes (MacDonald, Socransky and Gibbons, 1963; Socransky and Gibbons, 1965; Sundqvist *et al.*, 1979; Mayrand *et al.*, 1980). It is not known how the bacteria evade the antimicrobial action of the leukocytes. However, the results reported here suggest that some *Bacteroides* strains may degrade immunoglobulins and components of complement, known to be important in the phagocytosis and subsequent killing of bacteria by polymorphonuclear leukocytes. We have previously reported that the tissue fluid of sites in guinea pigs infected with *B. gingivalis* strain W83 contained no C3 and only traces of immunoglobulins (Sundqvist *et al.*, 1984).

Our findings that IgG and IgM are degraded by *B. gingivalis* and *B. intermedius* are in accord with previous results indicating that black-pigmented *Bacteroides* have such activity (Werner and Müller, 1971; Kilian, 1981; Sundqvist *et al.*, 1984). We could not, however, confirm that *B. asaccharolyticus* degrades these immunoglobulins (Werner and Müller, 1971; Kilian, 1981).

It is probable that most of the strains used in this study activated complement by physiological pathways. However, *B. gingivalis*, *B. intermedius* and *B. endodontalis* also caused a degradation of C3 fragments. Most of the *B. gingivalis* strains degraded C3 as well as C5 until fragments could no longer be detected with anti-C3, anti-C3c, anti C3d or anti-C5 sera. This degradation was probably independent of complement activation because C3 was degraded in human serum in which the classical and alternative pathways of complement activation were blocked by depletion of C4 and Factor B.

Complement components C3 and C5 of human serum have previously been demonstrated to be cleaved into biologically active fragments by proteinases from *S. marcescens* and a β -haemolytic streptococcus (Ward *et al.*, 1973). An elastase from *P. aeruginosa* has been shown to destroy the complement-dependent haemolytic activity of serum and the phagocytic activity of leukocytes (Schultz and Miller, 1974). *B. gingivalis* enzymes degrade not only C3, C5 and immunoglobulins of human serum but also albumin, haptoglobulin, haemopexin, transferrin and the proteinase inhibitors α -1-antitrypsin and α -2-macroglobulin (Carlsson, Höfling and Sundqvist, 1984; Carlsson *et al.*, 1984). These characteristics may explain the high pathogenic potential of *B. gingivalis* (McDonald *et al.*, 1963; Mayrand *et al.*, 1980; van Steenberg *et al.*, 1982; Sundqvist *et al.*, 1984). By degrading complement and immunoglobulin, *B. gingivalis* may evade the phagocytic host defence. The capacity to degrade the plasma proteinase inhibitors may remove those plasma proteins aimed at protecting the tissue against proteinases released by phagocytes and bacterial cells. Also, by degrading the haem-binding plasma proteins haemopexin and albumin, *B. gingivalis* may support the growth of haem-requiring organisms at infected sites.

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REFERENCES

- Carlsson J, Herrmann B F, Höfling J F, Sundqvist G K 1984 Degradation of the human proteinase inhibitors alpha-1-antitrypsin and alpha-2-macroglobulin by *Bacteroides gingivalis*. *Infection and Immunity* 43:644-648.
- Carlsson J, Höfling J F, Sundqvist G K 1984 Degradation of albumin, haemopexin, haptoglobin and transferrin by black-pigmented *Bacteroides* species. *Journal of Medical Microbiology* 18:39-46.
- Fong J S C, Renaud L 1978 A hemolytic plate method for alternate-pathway complement activity assay. *American Journal of Clinical Pathology* 69:156-160.
- Ganrot P O 1972 Crossed immunoelectrophoresis. *Scandinavian Journal of Clinical Laboratory Investigation Suppl* 124:39-47.
- Holdeman L V, Cato E P, Moore W E C 1977 *Anaerobe laboratory manual*, 4th edn. Virginia Polytechnic Institute and State University, Blacksburg VA, pp 144-148.
- Ingham H R, Sisson P R, Middleton R L, Narang H K, Codd A A, Selkon J B 1981 Phagocytosis and killing of bacteria in aerobic and anaerobic conditions. *Journal of Medical Microbiology* 14:391-399.
- Ingham H R, Sisson P R, Tharagonnet D, Selkon J B, Codd A A 1977 Inhibition of phagocytosis in vitro by obligate anaerobes. *Lancet* 2:1252:1254.
- Jones G R, Gemmell C G 1982 Impairment by *Bacteroides* species of opsonisation and phagocytosis of enterobacteria. *Journal of Medical Microbiology* 15:351-361.
- Kilian M 1981 Degradation of immunoglobulins A1, A2, and G by suspected principal periodontal pathogens. *Infection and Immunity* 34:757-765.
- Kilian M, Mestecky J, Schrohenloher R E 1979 Pathogenic species of the genus *Haemophilus*

- and *Streptococcus pneumoniae* produce immunoglobulin A1 protease. *Infection and Immunity* 26:143-149.
- Lange K 1962 Über den immunologischen Mechanismus der akuten und der chronischen Glomerulonephritis. In: Wollheim E (ed) *Glomeruläre und tubuläre Nierenerkrankungen*. Georg Thieme Verlag, Stuttgart, pp 232-245.
- Laurell C-B 1972 Electroimmuno assay. *Scandinavian Journal of Clinical Laboratory Investigation Suppl* 124:21-37.
- Löfgren S, Tärnvik A, Carlsson J 1980 Demonstration of opsonizing antibodies to *Francisella tularensis* by leukocyte chemiluminescence. *Infection and Immunity* 29:329-334.
- MacDonald J B, Socransky S S, Gibbons R J 1963 Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. *Journal of Dental Research* 42 (Suppl): 529-544.
- Male C J 1979 Immunoglobulin A1 protease production by *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Infection and Immunity* 26:254-261.
- Mayrand D, McBride B C, Edwards T, Jensen S 1980 Characterization of *Bacteroides asaccharolyticus* and *B. melaninogenicus* oral isolates. *Canadian Journal of Microbiology* 26:1178:1183.
- Neidhardt F C, Bloch P L, Smith D F 1974 Culture medium for enterobacteria. *Journal of Bacteriology* 119:736:747.
- Plaut A G, Gilbert J V, Artenstein M S, Capra J D 1975 *Neisseria gonorrhoeae* and *Neisseria meningitidis*: extracellular enzyme cleaves human immunoglobulin A. *Science* 190:1103-1105.
- Schultz D R, Miller K D 1974 Elastase of *Pseudomonas aeruginosa*: Inactivation of complement components and complement-derived chemotactic and phagocytic factors. *Infection and Immunity* 10:128-135.
- Socransky S S, Gibbons R J 1965 Required role of *Bacteroides melaninogenicus* in mixed anaerobic infections. *Journal of Infectious Disease* 115:247-253.
- Sundqvist G K, Eckerbom M I, Larsson Å P, Sjögren U T 1979 Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. *Infection and Immunity* 25:685-693.
- Sundqvist G K, Carlsson J, Herrmann B F, Höfling J F, Väättäin A 1984 Degradation in vivo of the C3 protein of guinea-pig complement by a pathogenic strain of *Bacteroides gingivalis*. *Scandinavian Journal of Dental Research* 92:14-24.
- Tofte R W, Peterson P K, Schmeling D, Bracke J, Kim Y, Quie P G 1980 Opsonization of four *Bacteroides* species: role of the classical complement pathway and immunoglobulin. *Infection and Immunity* 27:784-792.
- van Steenberg T J M, Kastelein P, Touw J J A, De Graaff J 1982 Virulence of black-pigmented *Bacteroides* strains from periodontal pockets and other sites in experimentally induced skin lesions in mice. *Journal of Periodontal Research* 17:41-49.
- Ward P A, Chapitis J, Conroy M C, Lepow I H 1973 Generation by bacterial proteinases of leukotactic factors from human serum, and human C3 and C5. *The Journal of Immunology* 110:1003-1009.
- Werner H, Müller H E 1971 Immunelektrophoretische Untersuchungen über die Einwirkung von *Bacteroides*-, *Fusobacterium*-, *Leptotrichia*- und *Spaerophorus*-Arten auf menschliche Plasmaproteine. *Zentralblatt für Bakteriologie I Abt. Orig.* 216:96-113.