INFECTION AND IMMUNITY, Feb. 1979, p. 532-539
0019-9567/79/02-0532/08$02.00/0

Vol. 23, No. 2

Group and Type Antigens of *Capnocytophaga*

R. H. STEVENS, B. F. HAMMOND,* AND C. H. LAI

Department of Microbiology, The Center for Oral Health Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received for publication 19 September 1978

Group-specific and type-specific antigens have been identified and purified from sonic extracts of *Capnocytophaga*. The group-specific antigen, which was purified by affinity chromatography, was found to be identical to an antigen present in all 26 strains tested. The antigen is sensitive to trypsin, sodium dodecyl sulfate and heat labile, and composed predominantly (55%) of protein. The type-specific antigen, which was obtained by preparative immunoelectrophoresis, was found to be present in only 3 of the 26 strains tested. This antigen was resistant to heat, trypsin, and sodium dodecyl sulfate and was primarily composed of carbohydrate (47% phenol-sulfuric acid-positive material, 8% amino sugar). Agglutination and fluorescent antibody data suggest that both the group- and type-specific antigens reside on the cell surface.

Recent studies have characterized the physiology and morphology of a proposed new genus of bacteria previously referred to as *Bacteroides ochraceus*: *Capnocytophaga* (E. R. Leadbetter, S. C. Holt, and S. S. Socransky, Arch. Mikrobiol., in press; S. C. Holt, E. R. Leadbetter, J. L. Simpson, E. D. Savitt, and S. S. Socransky, Arch. Mikrobiol., in press; S. S. Socransky, S. C. Holt, E. R. Leadbetter, A. C. R. Tanner, E. Savitt, and B. F. Hammond, Arch. Mikrobiol., in press). Much interest has been shown in this organism, since (i) it has been shown to be a predominant cultivable organism in the advancing front of periodontal lesions (12, 13) and (ii) it was shown to be capable of causing periodontal disease, including bone loss, when implanted into gnotobiotic rats (J. T. Irving, S. S. Socransky, M. G. Newman, and E. Savitt, J. Dent. Res. **55**:A783, 1976).

The distinguishing features of this goup were reported to be that they were gram-negative flexible rods with a gliding motility and a fermentative-type metabolism that requires CO_2 and were oxidase and catalase negative. In contrast, other known groups of gliding bacteria are primarily obligate aerobes that carry out a strictly respiratory metabolism and are oxidase and catalase positive. Further definition of *Capnocytophaga* as a group, and the relationship of the strains within the group, could be aided by serological studies. In addition, although evidence suggests that these organisms may be potentially pathogenic in humans, and clearly can cause disease in rats, a mechanism for their virulence has not as yet been proposed. Antigenic analysis may identify cellular components that could contribute to the organism's virulence.

Both taxonomic considerations and the possible significance of identifying and characterizing any virulence-associated cell components suggest the value of a thorough investigation of the antigenic composition of the *Capnocytophaga*. To date, there is little information available concerning the serological relationships or antigenic composition of the existing *Capnocytophaga* isolates.

This study was conducted to examine these relationships and to identify antigenic components of this group of organisms.

MATERIALS AND METHODS

Bacteria. Twenty-six strains of *Capnocytophaga* representing various clinical isolates from normal, periodontosis, and peridontitis patients, *Eikenella corrodens* 1073, *Bacteroides melaninogenicus* 381, *Selenomonas sputigena* 1304, and *Bacteroides oralis* ATCC 15930 were kindly provided by S. Socransky. Cultures of *Myxococcus fulvus* and *Sporocytophaga myxococcoides* were supplied by E. R. Leadbetter. *Bacteroides ochraceus* ATCC 27872 was obtained directly from the American Type Culture Collection.

Growth conditions. *Capnocytophaga* strains, *B. ochraceus*, and *S. sputigena* were grown in Trypticase soy broth (BBL) supplemented with hemin (5 mg/liter) and $NaHCO_3$ (0.1%, wt/vol). Incubation was at 37°C overnight in H_2 and CO_2 GasPak anaerobic jars (BBL). *B. melaninogenicus* and *B. oralis* were grown under similar conditions with the addition of menadione (0.1 mg/liter) to the medium. *E. corrodens* was grown anaerobically in an H_2-CO_2 atmosphere at 37°C in a medium composed of: Trypticase, 0.1%; Phytone, 0.3%; sodium formate, 0.1%; NaCl, 0.4%; KNO_3, 0.1%; and hemin, 0.3 mg/100 ml. *M. fulvus* was

grown aerobically at 30°C in 1% Tryptone–0.05 M sodium phosphate (pH 7.0). *S. myxococcoides* was grown aerobically at 30°C in a minimal salts medium composed of: $NaNO_3$, 0.1%; $CaCl_2 \cdot 2H_2O$, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.04%; $FeCl_3$, 0.002%; K_2HPO, 0.1 M; and glucose, 1%.

Cell preparation. Cells were harvested (Sorvall GSA rotor, 16,319 × *g* for 10 min, or Sharples continuous-flow centrifuge for larger volumes of cells), washed once in 0.85% NaCl, and twice in distilled water. The final washed pellet was resuspended in a minimal volume of distilled water to produce a dense cell suspension representing a 20-fold concentration of the cells.

Antigen extraction. The washed cells were sonically disrupted in an ice bath for 10 min with an ultrasonic disintegrator (MSE Inc.). The resulting sonic extract (SE) was clarified (12,062 × *g* for 10 min) and dialyzed against distilled water in a cold room overnight. After dialysis, the SE was lyophilized. In this manner, 2,200 mg (dry weight) of SE was obtained from 30 liters of broth culture of *Capnocytophaga* strain 67-51, and 125 mg was obtained from 1.5 liters of strain 4. Sonic extracts of other organisms were produced in smaller quantities, since these extracts were used only for antigen detection and not needed in the larger amounts required for antigen purification.

G antigen purification. Group-specific (G) antigen was purified as follows. To prepare affinity columns, 4 g of cyanogen bromide-activated Sepharose 4B (Sigma) was swollen in 800 ml of cold 1 mM HCl. The swollen beads were washed with 600 ml of cold BBS (0.618% boric acid, 0.954% sodium borate, 0.438% NaCl, pH 8.5) and added to 100 mg (dry weight) of rabbit anti-*Capnocytophaga* (strain 4) immunoglobulin G (obtained as described below). The mixture was allowed to incubate in the cold with slow, end-over-end mixing for 20 h, after which 0.2 ml of ethanolamine (0.05 M, pH 9) was added and mixed gently for 10 min. The resulting slurry was washed successively with cold BBS (300 ml), acetic acid (0.1 M, 100 ml), and finally again with BBS (400 ml). No 280-nm absorbing material could now be detected in the washes. The slurry was poured into a 30-ml syringe plugged with glass wool and equilibrated in the cold with PBS (0.765% NaCl, 0.0724% Na_2HPO_4, 0.021% KH_2PO_4, pH 7.2). *Capnocytophaga* strain 67-51 SE (100 mg, dry weight) was suspended in 9 ml of PBS, reclarified, and applied to the affinity column. Fractions (2 ml each) were monitored for absorbance at 280 nm, and peak fractions, representing nonbound material, were pooled. The column was washed for approximately 20 fractions after the optical density at 280 nm was equal to zero. Bound material was desorbed from the column using 3 M KSCN in PBS (4, 6). After addition of the desorbing buffer, fractions were again monitored for adsorption at 280 nm. Peak fractions were again pooled. The peaks representing bound and nonbound material were dialyzed in the cold against distilled water and lyophilized. The lyophilized material recovered from 20 such passages of SE through an affinity column was pooled. Total recovery of the bound material was 35 mg or 1.75% of the SE used for antigen purification.

T-antigen purification. Type-specific (T) antigen was purified as follows. A 6-mm-thick vertical slab gel consisting of 0.8% agarose in immunoelectrophoresis (IEP) buffer (0.54% sodium barbital, 0.43% sodium acetate, pH 8.2) was cast in a vertical gel electrophoresis apparatus (EC Corp.). Upper and lower tanks were filled with IEP buffer, and the gel was subjected to pre-electrophoresis at 100 V (constant voltage) with a cooling water jacket. Polarity was such that the starting zone was at the anode and the direction of electrophoresis was towards the cathode. After 15 min of pre-electrophoresis, 1,500 μg of *Capnocytophaga* strain 4 SE was suspended in 150 μl of IEP buffer, mixed with 20 μl of tracking dye (0.1% methyl green, 33% glycerol), and applied to each of eight wells of the slab gel. The SE was subjected to electrophoresis for 4.5 h under the same conditions as in the pre-electrophoresis. After electrophoresis, the slab was carefully removed, and a single trough was cut in the gel adjacent and parallel to the first well. The trough was filled with rabbit antiserum prepared against *Capnocytophaga* strain 4, and the gel was allowed to incubate overnight in a refrigerator. The development of a single precipitation band was observed, and the appropriate strip of gel containing the antigen from wells 2 through 8 was collected. The antigen was extracted from the gel by three successive freeze-thaw cycles, followed by forcing the gel through an unfiltered syringe and finally through a glass wool-filtered syringe (10). The clear filtrate from eight such preparative IEP runs was pooled, dialyzed in the cold against distilled water, and then lyophilized.

Antisera. Antisera against *Capnocytophaga* strains 4 and 67-51 were produced in 3-kg female New Zealand rabbits. The vaccine was prepared by harvesting cells of the appropriate strain from the surface of 12 Trypticase soy agar plates supplemented with hemin and $NaHCO_3$. The cells were washed four times in sterile saline, suspended in 15 ml of sterile saline, and preserved with 2 drops of phenol. Protein was determined by Nesslerization and found to be at a concentration of 1,600 μg/ml. The rabbits received 0.2 ml of the vaccine three times a week for 2 weeks, then once a week thereafter. Antiserum that was to be used as the ligand in the affinity columns was fractionated according to the method of Herbert (8) and chromatographed using diethylaminoethyl-cellulose (Sigma) to obtain the immunoglobulin G fraction.

Immunological procedures. IEP and double-diffusion precipitin reactions were conducted according to the methods of Campbell et al. (2). Tandem one-dimensional IEP was carried out by loading a linear row of wells with antigen suspensions and applying a current of 17 mA for 4 h at 4°C. After electrophoresis, a trough was cut in the agarose gel parallel to the row of wells. The trough was then filled with antiserum. Precipitin bands were visible after overnight incubation at 4°C. Agglutination tests were carried out as follows. Overnight broth cultures of *Capnocytophaga* strains 4, 35, and 111 were Formalin fixed (1% [vol/vol] Formalin, 2 h at room temperature), harvested, washed twice in PBS, and finally suspended in PBS at an optical density of 2.0 at 600 nm. Serial dilutions of antiserum to either strain 4 or 67-51 were carried out in a multiwell dilution tray (NUNC) using PBS. The cell suspension (50 μl) was added to 50 μl of each

serum dilution. Trays were incubated at 37°C for 2 h, then overnight at 4°C.

For fluorescent antibody analysis, 10-ml overnight broth cultures of strains 4, 35, 111, 67-51, and 21-54 were each washed once in buffer (0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2) and then suspended in one-half volume in the same buffer. A smear of each suspension was heat fixed or fixed in 95% methanol. Undiluted and appropriately diluted rabbit antisera to strain 4 and 67-51 were applied to each smear and allowed to incubate at room temperature for 30 min. In some cases antiserum that was previously absorbed with G antigen was used. Excess antiserum was removed by repeated washings in buffer. Fluorescein isothiocyanate-conjugated goat antiserum to rabbit immunoglobulin G (Miles Laboratories) was then applied to the smears, and after 30 min of incubation the excess antiserum was again removed by repeated washing. Controls were made using normal rabbit serum and antiserum absorbed with homologous cells. The cells were observed with a Zeiss fluorescent microscope equipped with a halogen illuminator (12 V, 100 W), a fluorescein isothiocyanate exciter filter (32 by 5.5 mm), types 50 and 53 barrier filters, and a BG 38 filter.

Chemical procedures. Protein determination was performed by the method of Lowry et al. (9). Total hexose was measured by the phenol-sulfuric acid reaction (5), and amino sugar was determined by the method of Aminoff et al. (1).

Partial characterization of antigens. (i) Heat lability. SE suspensions (10 mg/ml) of strains 4 and 67-51 were immersed in boiling water for 0, 0.5, 1.0, or 5.0 min. The heat-treated extracts, nontreated extracts, and nontreated G and T antigens were reacted with antiserum to strain 4. Loss of antigenicity (as determined by double-immunodiffusion precipitin reactions) after 0.5 min of exposure to 100°C was considered to be evidence of heat lability. Nonlabile material maintained its antigenicity after 5.0 min at 100°C.

(ii) SDS sensitivity. SE suspensions (10 mg/ml) of strains 4 and 25 were made 1% (wt/vol) with respect to sodium dodecyl sulfate (SDS) and incubated at 37°C with gentle shaking for 30 min. SDS-treated as well as untreated SE were dialyzed exhaustively against distilled water (initially at room temperature, subsequently at 4°C). The SDS-treated extracts, untreated extracts, and untreated reference suspensions of G and T antigens were tested with antiserum to strain 4 in double-immunoprecipitin reactions. The loss of a precipitin band after SDS treatment was considered to be evidence of SDS sensitivity.

(iii) Trypsin sensitivity. Lyophilized SE of strains 4 and 67-51 were suspended in buffer [0.05 M tris(hydroxymethyl)aminomethane-hydrochloride, 0.01 M $CaCl_2$, pH 8.1] at 10 mg/ml. Trypsin (Sigma, Type III) was added to a concentration of 500 μg/ml. The reaction mixtures (as well as appropriate controls for temperature, dilution, buffer, and trypsin inhibitor) were incubated for 5 h at 37°C. *N*-α-Tosyl-l-lysine chloromethane ketone hydrochloride trypsin inhibitor was then added (100 μl of a 0.4 mM solution), and the reaction mixture (as well as untreated SE and G- and T-antigen reference suspensions) was reacted with antiserum to strain 4 in double-immunodiffusion agarose gels. Antigen that failed to exhibit a precipitin band after trypsin treatment was considered to be trypsin sensitive.

(iv) Dialyzability. Purification of both G and T antigens involved exhaustive dialysis and recovery from dialyzer tubing having a molecular weight cutoff of 12,000.

RESULTS

Purification of *Capnocytophaga* G antigen. A typical chromatogram obtained by passing the SE of *Capnocytophaga* strain 67-51 through an affinity column possessing an anti-*Capnocytophaga* strain 4 ligand displayed two peaks (Fig. 1). Peak 1 represented nonbound material, and peak 2 indicated the bound material that was desorbed with the chaotrope KSCN. Figure 2 shows a cross-reacting antigen between strains 4 and 67-51. This antigen, which was present in the starting material (crude SE), adsorbed to the column and was desorbed with KSCN as the bound material. The cross-reacting antigen (peak 2 material) exhibited a single precipitin band when reacted with the homologous antiserum (Fig. 3). This single band shows identity with one of the three bands observed upon plating the strain 67-51 crude SE with the same

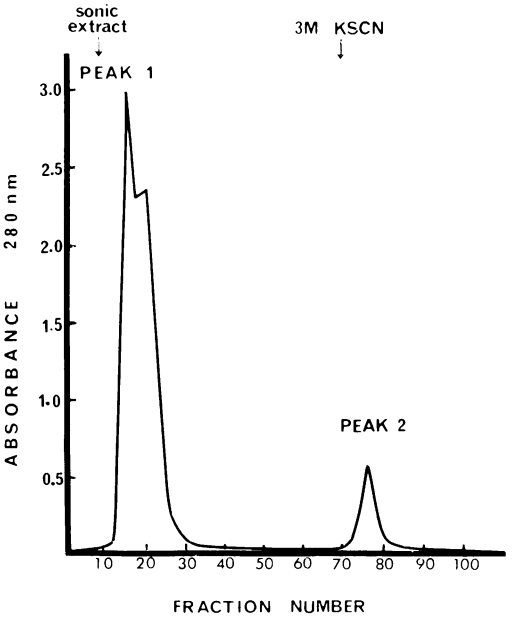

FIG. 1. *Affinity chromatography purification of Capnocytophaga group antigen. Strain 67-51 SE (100 mg, dry weight) was applied to an affinity column composed of rabbit anti-strain 4 immunoglobulin G bound to Sepharose 4B. After thorough washing of the column with PBS, the bound material was desorbed with 3 M KSCN in PBS. Fractions (2 ml) were assayed for absorbance at 280 nm.*

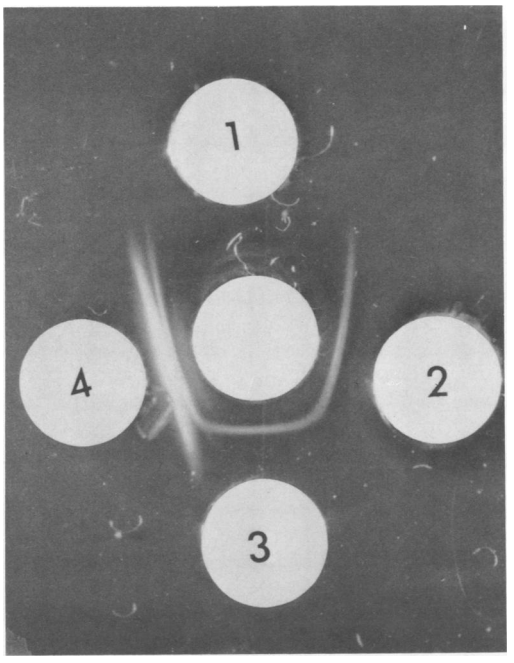

FIG. 2. *Immunodiffusion of material applied to and recovered from affinity column. The wells contain the following reagents: 1, peak 1 material from affinity column; 2, strain 67-51 SE applied to the column; 3, peak 2 material from column; 4, strain 4 SE; center, antiserum to strain 4.*

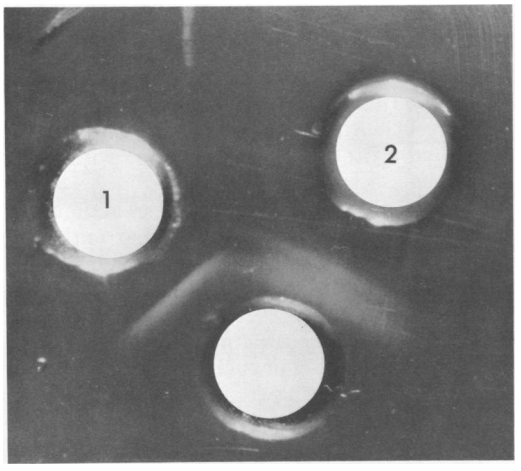

FIG. 3. *Immunodiffusion of peak 2 material from column. 1, Peak 2 material from column; 2, strain 67-51 SE applied to column; center, antiserum to strain 67-51.*

homologous antiserum. The antigenic purity of the peak 2 material is further supported by IEP analysis, in which a single, slightly cationic band was seen that appeared to correspond to one of the two bands detectable by IEP analysis of the strain 67-51 crude SE. The existence of a *Capnocytophaga* G antigen and the identity of the peak 2 material with this antigen are demonstrated in Fig. 4A and B. A single precipitin band showing identity between SE of 14 strains of *Capnocytophaga* and *B. ochraceus*, and the antigen isolated in peak 2 of the affinity column is evident. In Fig. 4B, there appears to be a partial identity reaction between the SE of strains 4, 35, and 111 with the peak 2 (G antigen) material. This appearance can be shown to be due to the superimposition of a precipitin band of a type antigen over the precipitin band formed by the G antigen (Fig. 5). Here it is apparent that, with the application of an electric potential, the two precipitin bands formed by the G and T antigens can be clearly differentiated. In all, 26 *Capnocytophaga* strains were examined, and all were

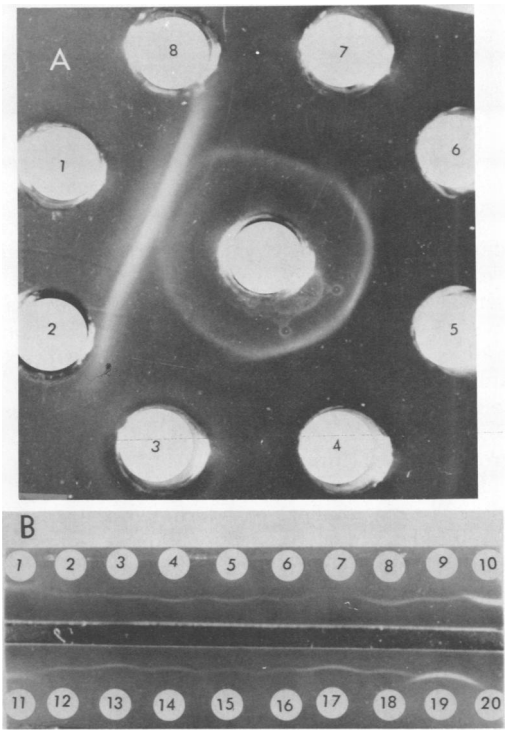

FIG. 4. *Immunodiffusion of peak 2 material from column, SE of B. ochraceus, and 14 strains of Capnocytophaga. (A) Wells contain: 1, 4 SE; 2, peak 2 material from column; 3, 67-51 SE; 4, 21-55 SE; 5, 61-92 SE; 6, 44 SE; 7, 120 SE; and 8, B. ochraceus SE; center, antiserum to strain 4. (B) Wells contain: 1, 4 SE; 2, 67-51 SE; 3, peak 2 material from column; 4, 30N-51 SE; 5, 30-11 SE; 6, 120 SE; 7, B. ochraceus SE; 8, 6 SE; 9, 21-55 SE; 10, 35 SE; 11, 4 SE; 12, 67-51 SE; 13, peak 2 material from column; 14, 67-47 SE; 15, 34-10 SE; 16, 44 SE; 17, 61-92 SE; 18, 25 SE; 19, 111 SE; 20, uninoculated broth. Trough contains antiserum to strain 4.*

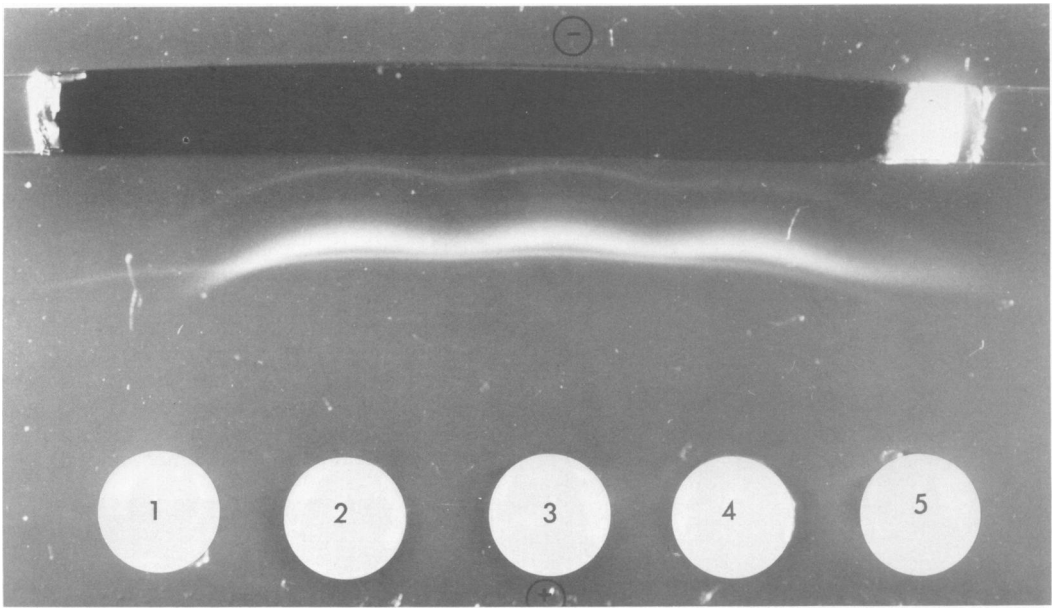

FIG. 5. *Tandem one-dimensional IEP of peak 2 material from column (well 1), strain 4 SE (well 2), strain 35 SE (well 3), strain 111 SE (well 4), and antigen isolated by preparative IEP (well 5). Trough contains antiserum to strain 4. Cathode was to the top.*

shown to possess the G antigen. Futhermore, we were unable to detect any cross-reacting antigen between *Capnocytophaga* and any representatives of other groups of gliding bacteria (*E. corrodens, M. fulvus,* and *S. myxococcoides*). Examination of other gram-negative organisms often found in dental plaque (*B. melaninogenicus, S. sputigina, B. oralis*) also failed to reveal any cross-reacting antigens.

Purification of *Capnocytophaga* T antigen. The material extracted from an agarose gel exhibited a single band upon double-immunodiffusion plating with the homologous (strain 4) antiserum (Fig. 6). This plate further demonstrates an identity reaction between the strain 4 antigen extracted from the gel and one of the three detectable antigens in the strain 4 crude SE. IEP analysis of the purified antigen (Fig. 7) revealed a single cationic band corresponding to the more cationic of two bands detected in the crude strain 4 SE.

The relationship of the antigen obtained by preparative IEP to the various *Capnocytophaga* strains is shown in Fig. 5 and 8. Four common antigens appear to be shared by strains 4, 35, and 111 (Fig. 5). A thick, diffuse cationic band shows an identity reaction with the antigen extracted from the preparative IEP gel. In contrast, the *Capnocytophaga* G antigen appears to be identical to the sharper, less cationic band. Whereas the antigen obtained from the preparative IEP is clearly common to strains 4, 35, and 111 (Fig. 5), the immunodiffusion pattern (Fig. 8) suggests that it is not the G antigen and not a component of any other *Capnocytophaga* strain examined.

Agglutination studies revealed that antiserum prepared against *Capnocytophaga* strain 4 had agglutinin titers of 2^8 when tested against strains 4, 35, or 111. Antiserum prepared against strain 67-51 had a titer of 2^7 when reacted with strain 4 cells.

Indirect fluorescent antibody reactions are shown in Table 1. It was found that antiserum to strain 4 gave equally intense fluorescence when reacted with strains 4, 35, or 111, regardless of whether the cells were heat fixed or methanol fixed. The fluorescence appeared as an intense, continuous outlining of each cell. The pattern of fluorescence resulting from reacting antiserum to strain 4 with cells of strain 67-51 or 21-54 was somewhat different in that the methanol-fixed cells of these strains exhibited a less intense fluorescence at points all along the cell periphery. The heat-fixed cells of strains 67-51 and 21-54 gave yet a less intense reaction with the same antiserum. Absorption of the antiserum to strain 4 with the G antigen completely eliminated any fluorescent labeling with strains 67-51 or 21-54, but did not affect the labeling of strains 4, 35, or 111. Antiserum to strain 67-51 reacted weakly to both heat-fixed and methanol-fixed cells of

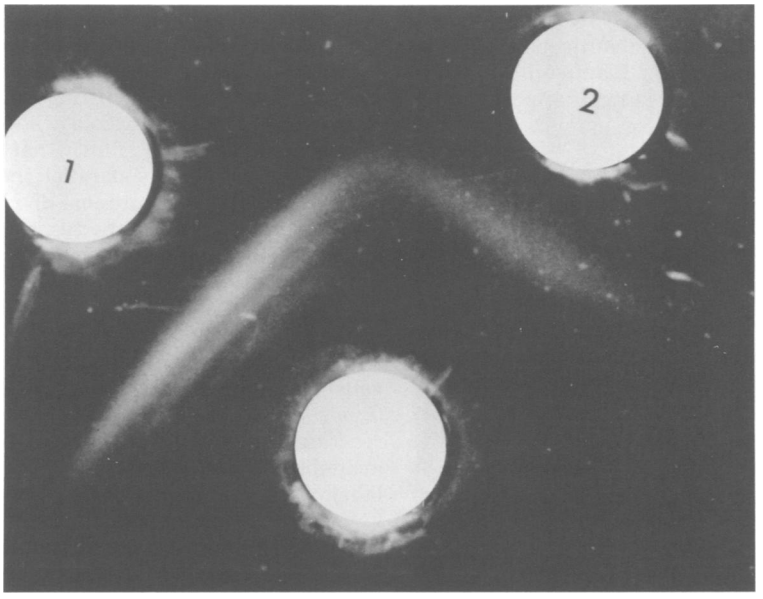

FIG. 6. *Immunodiffusion of T antigen. Well 1, strain 4 SE; well 2, T antigen isolated from strain 4 by preparative IEP; center well, antiserum to strain 4.*

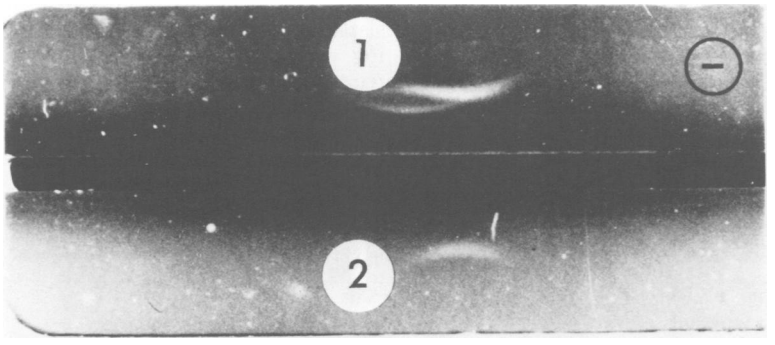

FIG. 7. *IEP of strain 4 SE (well 1) and T antigen (well 2). Trough contains antiserum to strain 4. Cathode is to the right.*

strains 4, 35, and 111, but reacted strongly to cells of strains 67-51 and 21-54 regardless of whether they were heat or methanol fixed. Preimmunized rabbit sera and antisera absorbed with homologous cells gave no fluorescent reaction with cells of any of the strains tested.

Preliminary characterization of the G antigen (Table 2) reveals a material that is composed primarily of protein, and is heat labile and sensitive to trypsin and SDS. In contrast, the T antigen appears to be predominantly carbohydrate, and is resistant to treatment with heat, trypsin, or SDS.

DISCUSSION

The data presented in this study support the contention that the *Capnocytophaga* represent an interrelated group of organisms. The physiological and morphological features that distinguish this group have been reported (Leadbetter et al., in press; Holt et al., in press; Socransky et al., in press).

The results of this study indicate that there is at least one antigen common to all the *Capnocytophaga* strains tested. The existence of this *Capnocytophaga* G antigen establishes the serological relatedness of all the *Capnocytophaga* strains examined thus far. In addition, no cross-reaction could be detected in double-immunodiffusion tests between *Capnocytophaga* and any other organism tested except *B. ochraceus*. On the basis of this evidence, we propose that the G material may be the G antigen of the *Capnocytophaga*.

While the *Capnocytophaga* strains examined have at least one common antigen, they also have what appears to be a T antigen. Of the 26 strains examined, one T antigen appears to be common to strains 4, 35, and 111. It is not yet clear whether the presence of the T antigen correlates with any morphological or physiological characteristics that distinguish these three strains. Preliminary physiological tests of these strains suggests the possibility that the presence of the T antigen may parallel the criteria by which Socransky et al. propose dividing the *Capnocytophaga* into three species, thereby providing a species-specific antigen.

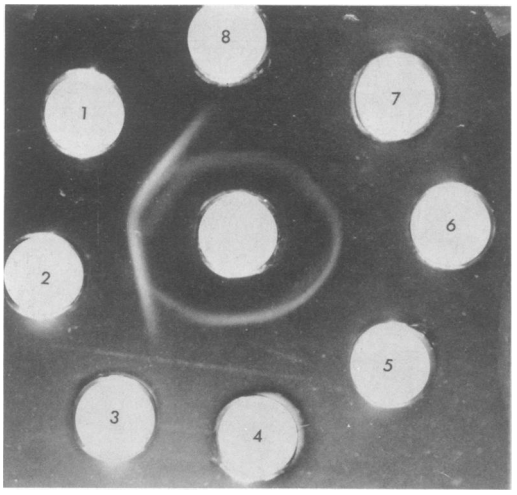

FIG. 8. *Immunodiffusion of T antigen isolated by preparative IEP and SE of seven strains of Capnocytophaga. Wells contain: 1, 4 SE; 2, T antigen; 3, 67-31 SE; 4, 21-55 SE; 5, 61-92 SE; 6, 44 SE; 7, 120 SE; 8, 30N-51 SE; center, antiserum to strain 4.*

The fluorescent antibody and agglutination data suggest that both the G and T antigens reside on the cell surface. The observation that antiserum to strain 4 (possessing G and T antigens) agglutinated strain 67-51 cells (possessing G but lacking T antigen) suggests that a cross-reacting antigen between strains 4 and 67-51 is available on the cell surface. The fluorescent labeling of cells of strains 67-51 and 21-54 with antiserum to strain 4 also indicates the presence of a cross-reacting surface antigen between these strains and strain 4. Since the purified G antigen was shown to be heat labile, it is of interest that the cross-reacting surface antigen appeared to be adversely affected by heat fixation since methanol fixation of strains 67-51 and 21-54 consistently resulted in a stronger fluorescent labeling with antiserum to strain 4 than did heat fixation. Furthermore, the observation that ab-

TABLE 1. *Fluorescent antibody reactions of Capnocytophaga strains*

Strain	Cell treatment	Antiserum[a]					
		Strain 4	4 absorbed with G antigen	Strain 67-51	NRS[b]	4 absorbed with 4 cells	67-51 absorbed with 67-51 cells
4	Heat	+++	+++	+	–	–	ND
	Methanol	+++	+++	+	–	–	ND
35	Heat	+++	+++	+	–	–	ND
	Methanol	+++	+++	+	–	–	ND
111	Heat	+++	+++	+	–	–	–
	Methanol	+++	+++	+	–	–	–
67-51	Heat	+	–	+++	–	–	–
	Methanol	++	–	+++	–	–	–
21-54	Heat	+	–	+++	–	–	–
	Methanol	++	–	+++	–	–	–

[a] +++, Strong fluorescence with distinct outline; ++, moderate fluorescence with distinct outline; +, weak fluorescence with faint outline; –, no fluorescence. ND, Not done.
[b] NRS, Normal rabbit serum.

TABLE 2. *Partial characterization of Capnocytophaga G and T antigens*

Antigen	Composition (%)			Heat lability	SDS sensitivity	Trypsin sensitivity	Dialyzability
	Protein	Carbohydrate	Amino sugar				
G	55	16	4	+	+	+	–
T	22	47	8	–	–	–	–

sorption of antiserum to strain 4 with purified G antigen eliminated fluorescent labeling with strains 67-51 and 21-54 strongly suggests that the G antigen is the cross-reacting surface antigen between strains 4, 67-51, and 21-54. With regard to the T antigen, it appeared that strains possessing the T antigen as well as the G antigen (i.e., strains 4, 35, and 111) exhibited a significantly stronger fluorescent labeling with antiserum to strain 4 than those strains lacking the T antigen (i.e., strains 67-51 and 21-54). This implies that the T antigen may be involved in the fluorescent antibody reaction and therefore may be a cell surface component.

Sonic oscillation is commonly considered a means of releasing soluble material from within the cell. However, it has been shown (11) that sonic disruption can solubilize components of the cell wall as well. It is, therefore, not inconsistent that we have solubilized cell surface components by means of sonic oscillation.

The localization of G and T antigens on cell surfaces is well documented for other organisms. For example, the M (type-specific) antigen and C (group-specific) antigen of the hemolytic streptococci have long been known to reside on the cell surface (3, 7, 14).

Our data suggest that the determinants of the *Capnocytophaga* G antigen are protein. From its slightly cationic behavior, the antigen, if protein, is likely to be basic, perhaps rich in arginine, lysine, or histidine.

In contrast, the T antigen determinants exhibit properties consistent with a carbohydrate. Its cationic behavior is somewhat puzzling, since the known amino sugars of bacterial cell walls are acetylated and would not account for the apparent positive charge. It is possible that the antigen, if polysaccharide, is linked to a basic cell wall component such as ethanolamine or a basic polypeptide. This could account for the antigen's cationic mobility.

Additional studies are currently in progress to establish more precisely the chemical nature of the G and T antigens. In conjunction with these studies, the purified antigens will be examined with regard to any biological activities that may contribute to virulence.

ACKNOWLEDGMENTS

This study was supported by a grant from the Colgate Palmolive Company and by Public Health Service grant DE-02623 from the National Institute of Dental Research..

The authors wish to acknowledge the competent technical assistance of Marilyn Darkes.

LITERATURE CITED

1. **Aminoff, D., W. T. J. Morgan, and W. M. Watkins.** 1952. Studies in immunochemistry. II. The action of dilute alkali on the N-acetyl hexosamines and the specific blood-group mucoids. Biochem. J. **51**:379–389.
2. **Campbell, D. H., S. G. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1963. Methods in immunology, p. 53–57 and 143–155. W. A. Benjamin, Inc., New York.
3. **Cole, R. M., and J. J. Hahn.** 1962. Cell wall replication in *Streptococcus pyogenes*. Science **135**:722–724.
4. **Dandliker, W. B., R. Alonso, V. A. de Saussure, F. Kierszenbaum, S. A. Levison, and H. C. Shapiro.** 1967. The effect of chaotropic ions on the dissociation of antigen-antibody complexes. Biochemistry **6**: 1460–1466.
5. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Roberts, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
6. **Fine, D. H., L. Tabak, and R. Stevens.** 1977. Affinity chromatography of antiserum to a gram-negative organism. J. Immunol. Methods **16**:91–96.
7. **Hahn, J. J., and R. N. Cole.** 1963. Streptococcal M antigen location and synthesis studied by immunofluorescence. J. Exp. Med. **118**:659–666.
8. **Herbert, G. A.** 1975. Improved salt fractionation of animal serums for immunofluorescence studies. J. Dent. Res. **55**(special issue A):A33–A37.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **McGrogan, M., and H. J. Raskas.** 1977. Species identification and genome mapping of cytoplasmic adenovirus type 2 RNAs synthesized late in infection. J. Virol. **23**: 240–249.
11. **Slade, H. D., and J. K. Vetter.** 1956. Studies on *Streptococcus pyogenes*. I. Observations on the microscopical and biological aspects of the disintegration and solubilization of a type 6 strain by sonic oscillation. J. Bacteriol. **71**:236–243.
12. **Slots, J.** 1976. The predominant cultivable organisms in juvenile periodontitis. Scand. J. Dent. Res. **84**:1–10.
13. **van Palenstein Helderman, W. H.** 1975. Total viable counts and differential count of *Vibrio* (*Campylobacter*) *sputorum*, *Fusobacterium nucleatum*, *Selenomonas sputigena*, *Bacteroides ochraceus*, and *Veillonella* in the inflamed and non-inflamed human gingival crevice. J. Periodont. Res. **10**:294–305.
14. **Willers, J. M., P. A. Deddish, and H. D. Slade.** 1968. Transformation of type polysaccharide antigen synthesis and hemolysin synthesis in streptococci. J. Bacteriol. **96**:1225–1230.