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Serology of Oral *Actinobacillus actinomycetemcomitans* and Serotype Distribution in Human Periodontal Disease

JOSEPH J. ZAMBON,* JØRGEN SLOTS, AND ROBERT J. GENCO

Department of Oral Biology and Periodontal Disease Clinical Research Center, State University of New York at Buffalo, Buffalo, New York 14226

Received 18 January 1983/Accepted 31 March 1983

Actinobacillus actinomycetemcomitans from the human oral cavity was serologically characterized with rabbit antisera to the type strain NCTC 9710; a number of reference strains, including Y4, ATCC 29522, ATCC 29523, ATCC 29524, NCTC 9709; and our own isolates representative of each of 10 biotypes. Using immunoabsorbed antisera, we identified three distinct serotypes by immunodiffusion and indirect immunofluorescence. Serotype a was represented by ATCC 29523 and SUNYaB 75; serotype b was represented by ATCC 29522 and Y4; and serotype c was represented by NCTC 9710 and SUNYaB 67. Indirect immunofluorescence revealed no reaction between the three *A. actinomycetemcomitans* serotype-specific antisera and 62 strains representing 23 major oral bacterial species. Distinct from the serotype antigens were at least one *A. actinomycetemcomitans* species common antigen and an antigen shared with other *Actinobacillus* species, *Haemophilus aphrophilus*, and *Haemophilus paraphrophilus*. All serotype a *A. actinomycetemcomitans* strains failed to ferment xylose, whereas all serotype b organisms fermented xylose. Serotype c included xylose-positive as well as xylose-negative strains. A total of 301 isolates of *A. actinomycetemcomitans* from the oral cavity of 74 subjects were serologically categorized by indirect immunofluorescence with serotype-specific rabbit antisera. Each patient harbored only one serotype of *A. actinomycetemcomitans*. Fourteen healthy subjects, five diabetics, and seventeen adult periodontitis patients exhibited serotypes a and b in approximately equal frequency, whereas serotype c was found less frequently. In contrast, in 29 localized juvenile periodontitis patients, the incidence of serotype b was approximately two times higher than that of serotypes a or c, suggesting a particularly high periodontopathic potential of *A. actinomycetemcomitans* serotype b strains. In subjects infected with *A. actinomycetemcomitans*, serum antibodies were detected to the serotype antigens, indicating that these antigens may play a role in the pathogenesis of periodontal disease.

Immunological studies of pathogenic bacteria have been highly valuable in implicating microorganisms and specific microbial components in the etiology of infectious diseases. Important examples include the incrimination of group A beta-hemolytic streptococci in human infections (15) and that of the polyribose phosphate moiety of capsular polysaccharide in the virulence of *Haemophilus influenzae* serotype b (33).

Actinobacillus actinomycetemcomitans is an oral gram-negative facultative organism, which is closely related to the *Haemophilus* group of organisms. This organism can cause severe human infections, including bacterial endocarditis (20, 21, 24, 31), thyroid gland abscess (2), urinary tract infection (30), brain abscess (13), and vertebral osteomyelitis (18). *A. actinomycetemcomitans* produces a potent endotoxin (10), a collagenase (J. Rozanis and J. Slots, J. Dent. Res., Abstr. Int. Assoc. Dent. Res. 1982, no. 275), a leukotoxin capable of lysing human polymorphonuclear leukocytes in vitro (1), and other cytotoxic products (7).

A. actinomycetemcomitans has been implicated in the pathogenesis of certain types of human periodontal disease. Patients with localized juvenile periodontitis frequently harbor *A. actinomycetemcomitans* in high numbers and develop serum and gingival crevicular fluid antibodies against the organism (3, 5, 26; P. A. Murray and R. J. Genco, J. Dent. Res. Special Issue A, Abstr. Am. Assoc. Dent. Res. 1980, no. 329). The elimination of *A. actinomycetemcomitans* from localized juvenile periodontitis lesions results in the resolution of the disease (27).

Few data are available on the serology of periodontal *A. actinomycetemcomitans*. Pulverer and Ko (23) identified six different anti-

gens and 24 different tube agglutination patterns among 100 strains of *A. actinomycetemcomitans* from oral and nonoral infections. They also detected species-common antigen(s) by the Ouchterlony gel diffusion technique. King and Tatum found that each of 33 *A. actinomycetemcomitans* strains examined by capillary tube precipitation contained one of three antigens (11), as well as antigens shared with *Haemophilus aphrophilus* and other *Haemophilus* species.

In view of the importance of *A. actinomycetemcomitans* in human periodontal disease, it was considered essential to establish the antigenic relationship between oral strains of *A. actinomycetemcomitans* and between *A. actinomycetemcomitans* and various closely and distantly related oral bacterial species. Also determined was the distribution of the serotypes of *A. actinomycetemcomitans* in different ecological sites within the oral cavity and among individuals with different types of periodontal disease.

(Portions of this work were previously presented; [J. J. Zambon, R. J. Genco, and J. Slots, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C240, p. 311].)

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacteria used to produce rabbit antisera included 16 strains of *A. actinomycetemcomitans*, including the type strain, type strains of *A. equuli* and *A. seminis*, one strain each of *A. lignieresii* and *A. suis*, three strains of *H. paraphrophilus*, and four strains of *H. aphrophilus* (Table 1). A total of 301 well-classified isolates of *A. actinomycetemcomitans* and 62 representative strains from 23 oral bacterial species obtained from our stock culture collection were examined by indirect immunofluorescence for reactivity with immunoabsorbed antisera to *A. actinomycetemcomitans* (Table 2). The source and characteristics of the *A. actinomycetemcomitans* isolates tested are described in detail elsewhere (J. J. Zambon, L. A. Christersson, and J. Slots, J. Periodontol., in press). It should be pointed out that although some investigators have reported a few strains of *A. actinomycetemcomitans* which were catalase negative (29), this study includes only catalase-producing organisms (6, 11, 23, 25).

To prepare the bacterial cells for immunological procedures, we grew strains of *Actinobacillus* and *Haemophilus* species to late-logarithmic or early-stationary phase in NIH thioglycolate liquid media (Difco Laboratories, Detroit, Mich.). V-factor-requiring *Haemophilus* species were grown on chocolate agar for 72 h. Most strains of the other oral species were grown in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.). All strains were grown at 37°C in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 85% N_2, 10% H_2, and 5% CO_2. The bacteria grown in liquid media were harvested by centrifugation and washed three times in phosphate-buffered saline (PBS; pH 7.2). The strains grown on agar plates were harvested with a platinum loop and also washed three times in PBS.

Antisera production. Whole bacterial cells to be used as immunogens were suspended in sterile saline to a concentration of 10 mg (wet weight) per ml. Eight-week-old female New Zealand white rabbits weighing 4 kg received 12 intravenous injections of 1.0 ml of bacterial suspensions via the marginal ear vein by the protocol of McCarty and Lancefield (16). For each strain, antisera were produced in two or more rabbits. Trial blood samples were obtained from the central ear artery, and the antibody titers were determined by immunodiffusion. Once a satisfactory antibody titer had been obtained, the rabbits were exsanguinated by cardiac puncture. The antisera were heated to 56°C for 30 min and stored in small portions at −76°C until used

Immune absorption. Whole bacterial cells (100 mg [wet weight]) were added to 1 ml of rabbit antisera and placed in a shaker at 37°C for 1 h and then at 4°C for 12 h. The mixture was centrifuged at 16,000 × *g* for 60 min, and the antisera supernatant was removed. The absorption was repeated, and the absorbed immune antisera were stored at −76°C. The serotype specificity of the immunoabsorbed antisera was confirmed by the absence of reactivity with bacterial strains from the other *A. actinomycetemcomitans* serotypes by immunodiffusion and indirect immunofluorescence assays.

TABLE 1. *Actinobacillus* and *Haemophilus* species used in the preparation of rabbit antisera and bacterial sonic extracts

Organism	Strain	Source
A. actinomycetemcomitans	29522	ATCC[a]
	29523	ATCC
	29524	ATCC
	9709	NCTC[b]
	9710[c]	NCTC
	Y4	Socransky[d]
	1	SUNYaB[e]
	15	SUNYaB
	27	SUNYaB
	29	SUNYaB
	32	SUNYaB
	39	SUNYaB
	42	SUNYaB
	67	SUNYaB
	75	SUNYaB
	85	SUNYaB
A. equuli	19392[c]	ATCC
A. lignieresii	19393	ATCC
A. seminis	15768[c]	ATCC
A. suis	15557	ATCC
H. aphrophilus	13252	ATCC
	19415[c]	ATCC
	5906	NCTC
	5908	NCTC
H. paraphrophilus	29240	ATCC
	29241[c]	ATCC
	29242	ATCC

[a] American Type Culture Collection, Rockville, Md.

[b] National Collection of Type Cultures, London, United Kingdom.

[c] Type strain.

[d] S. S. Socransky, Forsyth Dental Center, Boston, Mass.

[e] State University of New York at Buffalo.

TABLE 2. Oral microorganisms examined by indirect immunofluorescence for the presence of cross-reacting antigens with serotype-specific rabbit antisera to *A. actinomycetemcomitans*

Organism	No. of strains	Source
Actinobacillus equuli	1	ATCC[a] 19392[b]
Actinobacillus lignieresii	1	ATCC 19393
Actinobacillus suis	1	ATCC 15557
Actinomyces israelii	1	SUNYaB
Actinomyces viscosus	1	SUNYaB
Arachnia propionica	1	SUNYaB
Bacteronemia matruchotii	1	SUNYaB
Bacteroides gingivalis	3	SUNYaB
Bacteroides melaninogenicus subsp. *intermedius*	2	SUNYaB
Bacteroides melaninogenicus subsp. *levii*	1	SUNYaB
Bacteroides melaninogenicus subsp. *melaninogenicus*	3	SUNYaB
Campylobacter sputorum	1	SUNYaB
Capnocytophaga ochracea	3	SUNYaB
Fusobacterium nucleatum	2	SUNYaB
Haemophilus aegyptius	1	ATCC 11116
Haemophilus aphrophilus	4	ATCC 13252 ATCC 19415[b] NCTC[c] 5906 NCTC 5908
Haemophilus ducreyi	1	ATCC 27722
Haemophilus influenzae	1	ATCC 19418
Haemophilus influenzae type a	1	ATCC e9006
Haemophilus influenzae type b	1	ATCC 9795
Haemophilus influenzae type c	1	ATCC 9007
Haemophilus influenzae type d	1	ATCC 9332
Haemophilus influenzae type e	1	ATCC 8142
Haemophilus influenzae type f	1	ATCC 9833
Haemophilus parahaemolyticus	4	ATCC 10014[b] ATCC 27088 ATCC 27089 ATCC 27090
Haemophilus parainfluenzae	2	ATCC 7901 ATCC 9796
Haemophilus paraphrohaemolyticus	1	ATCC 29237[b]
Haemophilus paraphrophilus	3	ATCC 29240 ATCC 29241[b] ATCC 29242
Streptococcus mitis	2	SUNYaB
Streptococcus mutans	2	SUNYaB
Veillonella parvula	13	SUNYaB

[a] American Type Culture Collection, Rockville, Md.

[b] Type strain.

[c] National Collection of Type Cultures, London, United Kingdom.

Sonic extraction of bacterial cell antigens. Whole bacterial cells (100 mg [wet weight]) were placed in 15 ml of PBS in an ice bath and sonicated (Sonifier cell disrupter, model 350; Bronson Sonic Power Co., Danbury, Conn.) at 25 W with a tapered microtip having an end diameter of 3 mm (model 419; Heat Systems Ultrasonics, Plainview, N.Y.) until greater than 95% of the cells were disrupted as determined by phase-contrast microscopy. Generally, 60 min of sonication was required. The sonic extracts were centrifuged at 12,000 × *g* for 30 min at 4°C, and the supernatants were removed, dialyzed overnight against distilled water, and lyophilized. The sonic extracts were then suspended to a protein concentration of 25 mg/ml as determined by the assay of Lowry et al. (12) and stored at −20°C until used.

Immunodiffusion. Immunodiffusion was carried out in 1.2% agarose (SeaKem ME; FMC Corp., Rockland, Maine) in 0.033 M Veronal buffer (pH 8.2) by the method of Ouchterlony (19). Relevant combinations of bacterial cell sonic extracts and rabbit immune sera were examined. The negative controls included rabbit preimmune sera.

Immunofluorescence. Indirect immunofluorescence was carried out essentially as described by Mouton et al. (17). Bacteria grown for 3 to 5 days on Trypticase soy blood agar (BBL Microbiology Systems, Cockeysville, Md.) were harvested with a platinum loop and placed in PBS to an optical density at 540 nm of 0.7. Ten microliters of the bacterial suspension was distributed onto prewashed glass slides and gently heat fixed. The slides were stored at room temperature until used.

Ten microliters of rabbit antisera at working titer (the highest twofold serial dilution still giving a brilliant fluorescence of the cell envelope) in PBS containing 0.05% Tween 20 (PBS-T) was placed on the bacterial smears for 10 min, rinsed with PBS-T, washed in PBS, and rinsed with distilled water. The slides were then incubated with 25 μl of goat anti-rabbit immunoglobulin G (IgG) conjugated with 25 μg of fluorescein isothiocyanate (isomer I BBL Microbiology Systems) per mg of protein and diluted 1:100 with PBS-T. The slides were rinsed, washed as before, and then mounted with glycerol in PBS (2:1 [vol/vol]; pH 9.0).

A Leitz Orthoplan microscope equipped for phase-contrast illumination and for incident-light fluorescence was used to examine the stained bacterial smears. The light source was an Osram HBO-200 mercury lamp with a BG filter and a dichroic 495-nm interference filter in its exciting pathway and a K510 suppression filter. Fluorescence was graded from 1+ to 4+; grades 3+ and 4+ were considered serologically positive reactions.

The immunofluorescent serotyping of *A. actinomycetemcomitans* strains was performed with serotype-specific rabbit antisera at working titer (generally 1:256 to 1:512), whereas representative strains of other oral microorganisms were examined for the presence of cross-reacting antigens by indirect immunofluorescence with serotype-specific antisera at the working titer concentrated four times (1:64 to 1:128).

RESULTS

In an initial agarose gel double-diffusion examination involving all combinations of the 16

A. actinomycetemcomitans strains, listed in Table 1 and the corresponding whole rabbit antisera, each *A. actinomycetemcomitans* strain was shown to contain as many as 12 antigens. However, it was also revealed that three distinct precipitin reaction patterns existed among *A. actinomycetemcomitans* strains. Immunoabsorption with whole cells of *A. actinomycetemcomitans*, which eliminated all but one precipitin line, clarified these serological patterns (Fig. 1). Antisera against *A. actinomycetemcomitans* 75 that was absorbed with whole cells of strains Y4 and 67 contained only one precipitating antibody to an antigen which was designated a. Likewise, antisera to strain Y4 absorbed with cells of strains 75 and 67 and antisera to strain 67 absorbed with strains Y4 and 75 define antigens b and c, respectively. Furthermore, the precipitin line formed by the homologous reaction of a specific antiserum with strain 75 bacterial sonic extract showed a reaction of nonidentify with that formed by b-specific antisera reacting with Y4 bacterial sonic extract. Similarily, we observed a reaction of nonidentity between the precipitin line formed by antisera a with strain 75 and that from the reaction of antisera c with strain 67. Finally, the b and c precipitin lines also showed nonidentify.

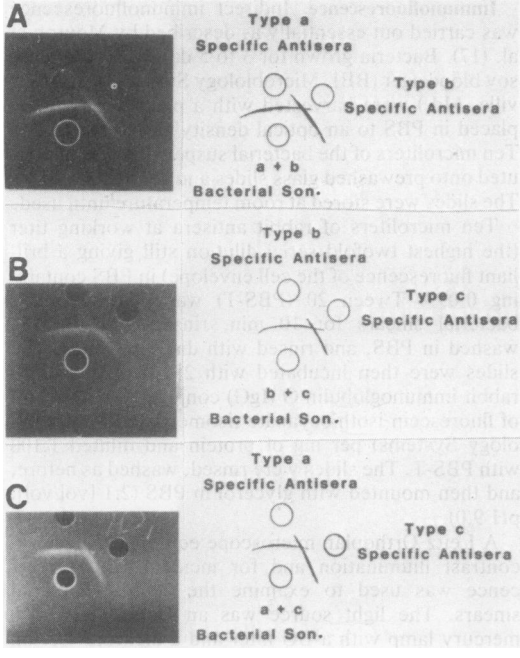

FIG. 1. Ouchterlony double diffusion showing serotype reactions of *A. actinomycetemcomitans*. (A) Serotype a-specific antisera (rabbit antisera to strain 75 absorbed with whole bacterial cells of strains Y4 and 67), serotype b-specific antisera (rabbit antisera to strain Y4 absorbed with whole cells of strain 67 and 75), and equal volumes of bacterial sonic extracts from strains 75 (type a) and Y4 (type b). (B) Serotype b-specific antisera, serotype c-specific antisera (rabbit antisera to strain 67 absorbed with whole cells of strains Y4 and 75), and equal volumes of bacterial sonic extracts from strains Y4 (type b) and 67 (type c). (C) Serotype a-specific antisera, serotype c-specific antisera, and equal volumes of serotypes a and c. The bacterial sonic extracts were at a protein concentration of 25 mg/ml.

Indirect immunofluorescence with immunoabsorbed antisera also revealed three serotypes of *A. actinomycetemcomitans*. Serotype a-specific antisera used at the working titer exhibited positive fluorescence with strain 75 but not with strains 67 or Y4. Also, serotype b- and serotype c-specific antisera yielded positive reactions with strains Y4 and 67, respectively, but no fluorescence with the heterologous strains. Serotaxonomy of the remaining reference strains by immunodiffusion and indirect immunofluorescence assays showed that the type strain NCTC 9710 and NCTC 9709 belonged to serotype c, that ATCC 29523 belonged to serotype a, and that ATCC 29522 and ATCC 29524 belonged to serotype b.

To determine whether the serotype-specific surface antigens of *A. actinomycetermcomitans* may be shared with other organisms from the human mouth, we examined a number of oral bacteria, including *Actinobacillus* and *Haemophilus* species, by indirect immunofluorescence, using serotype-specific antisera at four times the working titer (1:64 to 1:128). None of the organisms listed in Table 2 reacted with the *A. actinomycetemcomitans* serotype-specific antisera, indicating the unique occurrence of the serotype antigens of *A. actinomycetemcomitans*.

Figure 2 shows that several common antigens exist between *A. actinomycetemcomitans* and other members of the *Actinobacillus* genus. Whole antisera to *A. actinomycetemcomitans* strain 75 reacted with sonic extracts of Y4 and 67, as well as with *A. lignieresii*, *A. suis*, and *A. equuli* sonic extracts. Conversely, rabbit antisera to *A. suis* ATCC 15557 formed precipitin lines with bacterial sonic extracts of these same organisms, indicating the presence of common antigens. Agarose gel double-diffusion reactions using rabbit antisera to *A. equuli*, *A. lignieresii*, *A. seminis*, and five additional strains of *A. actinomycetemcomitans* (ATCC 29523, Y4, SUNYaB 15, SUNYaB 42, and SUNYaB 67) with bacterial sonic extracts of these same bacteria also revealed from one to three common precipitin bands.

In addition, common antigens existed between *A. actinomycetemcomitans* and the closely related species *H. aphrophilus*. Rabbit antisera to *A. actinomycetemcomitans* Y4 reacted with sonic extracts of *H. aphrophilus* and *A.*

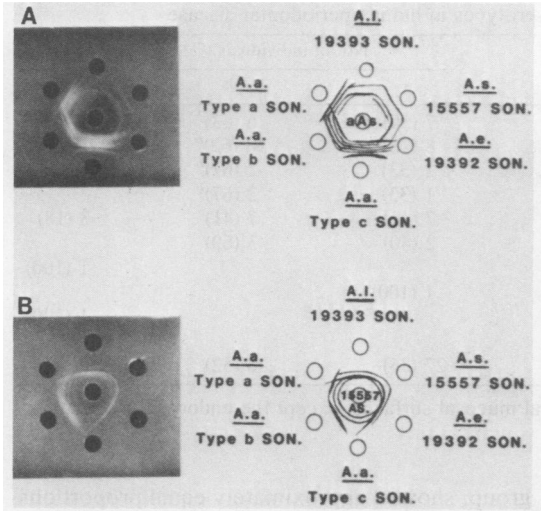

FIG. 2. Ouchterlony agarose gel diffusion reactions showing common antigens within the genus *Actinobacillus*. (A) Bacterial sonic extracts of *A. lignieresii* ATCC 19393, *A. suis* ATCC 15557, *A. equuli* ATCC 19392, *A. actinomycetemcomitans* serotype c (SUNYaB 67), *A. actinomycetemcomitans* serotype b (Y4), and *A. actinomycetemcomitans* serotype a (SUNYaB 75). The center well contains *A. actinomycetemcomitans* serotype a rabbit antisera. (B) Bacterial sonic extracts as in (A) with rabbit antisera to *A. suis* ATCC 15557 in the center well. The bacterial sonic extracts were utilized at a protein concentration of 25 mg/ml.

actinomycetemcomitans (Fig. 3A). Conversely, rabbit antisera to *H. aphrophilus* developed precipitin bands with *A. actinomycetemcomitans* and *H. aphrophilus* sonic extracts (Fig. 3B). Also evident was a precipitin band common to the *H. aphrophilus* strains, which was not shared by *A. actinomycetemcomitans*. Gel diffusion reactions showing common antigens were also seen when sonic extracts and rabbit antisera to *H. paraphrophilus* were used in place of *H. aphrophilus* (data not shown).

Patients who yielded cultivable *A. actinomycetemcomitans* generally exhibited serum antibodies to the serotype antigens. Figure 4A shows the precipitin reaction between serum from a patient harboring *A. actinomycetemcomitans* serotype a in microbial dental plaques, serotype a-specific rabbit antisera, and bacterial sonic extracts of strain 75 (serotype a). The precipitin bands between patient serum and strain 75 sonic extracts formed a reaction of identity with that found between serotype a-specific antisera and strain 75 sonic extracts. Likewise, the serum of a patient who was orally infected with *A. actinomycetemcomitans* serotype b revealed antibodies to the serotype b antigen, and subjects harboring the serotype c organisms possessed serum antibodies to the c antigen. No reaction was seen between the patient serum and the serotype-specific antigens from the two other serotypes. These results indicate the presence of antibodies to the serotype antigens in the patient serum and again show serological specificity among *A. actinomycetemcomitans* strains.

Table 3 demonstrates the distribution of *A. actinomycetemcomitans* serotypes in infected human subjects with various periodontal conditions. From 1 to 19 oral isolates of *A. actinomycetemcomitans* were serotyped per individual, and a total of 301 *A. actinomycetemcomitans* isolates were examined. Importantly, all *A. actinomycetemcomitans* study isolates reacted with one of the three serotype-specific antisera, suggesting that our immunological reagents are capable of detecting all oral *A. actinomycetemcomitans* and that if additional serotypes exist, as determined by our method, they are very rare. No patient harbored more than one serotype of *A. actinomycetemcomitans*. Of 14 healthy subjects, 7 yielded *A. actinomycetemcomitans* serotype a, 6 yielded serotype b, and 1 yielded serotype c. Twenty-nine localized juvenile periodontitis patients, three generalized juvenile periodontitis patients, and three postlocalized juvenile periodontitis patients exhibited

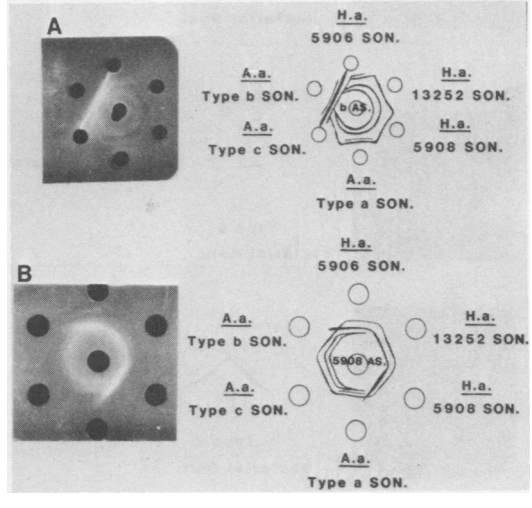

FIG. 3. Agarose gel double-diffusion reactions indicating cross-reactions between *A. actinomycetemcomitans* and *H. aphrophilus*. (A) Bacterial sonic extracts of *H. aphrophilus* NCTC 5906, *H. aphrophilus* ATCC 13252, *H. aphrophilus* NCTC 5908, *A. actinomycetemcomitans* serotype a (SUNYaB 75), *A. actinomycetemcomitans* serotype c (SUNYaB 67), and *A. actinomycetemcomitans* serotype b (Y4). The center well contains serotype b rabbit antisera. (B) Same as in (A) except that the center well contains rabbit antisera to *H. aphrophilus* NCTC 5908.

TABLE 3. *A. actinomycetemcomitans* serotypes in human periodontal disease

Patient category[a]	No. of subjects	No. of individuals (%) serotype:		
		a	b	c
Periodontally healthy	14	7 (50)	6 (43)	1 (7)
Localized juvenile periodontitis	29	8 (28)	18 (62)[b]	3 (10)
Generalized juvenile periodontitis	3	1 (33)	2 (67)[b]	
Postlocalized juvenile periodontitis	3	1 (33)	2 (67)[b]	
Adult periodontitis	17	7 (41)	7 (41)	3 (18)
Insulin-dependent diabetes mellitus	5	2 (40)	3 (60)	
Lymphoma	1			1 (100)
Dilantin gingival hyperplasia	1	1 (100)		
Bacterial endocarditis	1			1 (100)
Total	74	27 (36)	38 (52)	9 (12)

[a] All isolates originated in periodontal pockets and oral mucosal surfaces, except the endocarditis isolate.
[b] $P < 0.05$ as determined by chi-square analysis.

about a twofold-higher frequency of *A. actinomycetemcomitans* serotype b than of serotypes a and c combined. Again, c was a rare serotype in these patients. Diabetic subjects and adult periodontitis patients, similar to the healthy group, showed approximately equal proportions of serotypes a and b, whereas serotype c was found in only a few patients. On the other hand, one lymphoma patient and one bacterial endocarditis patient exhibited *A. actinomycetemcomitans* serotype c isolates, and one patient with dilantin hyperplasia yielded isolates of serotype a and, accordingly, showed no serotype b isolates.

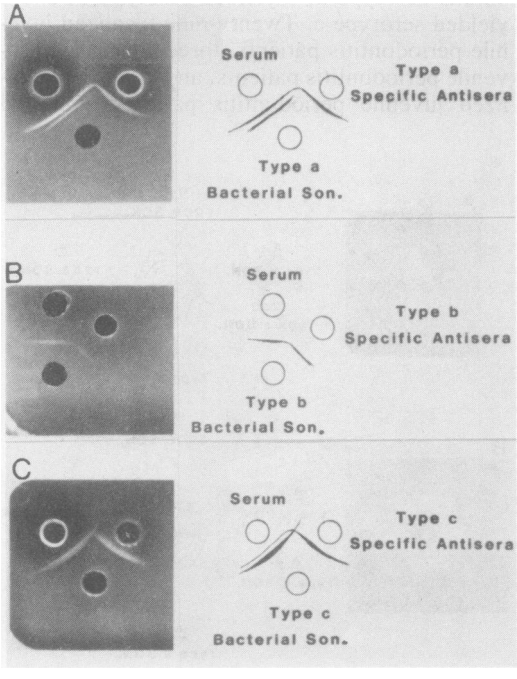

FIG. 4. Agarose gel double-diffusion reactions showing that patients who harbor oral *A. actinomycetemcomitans* develop serum antibodies against the serotype antigens. (A) Serum from a localized juvenile periodontitis patient, serotype a-specific rabbit antisera, serotype a bacterial sonic extract (strain 75). (B) Serum from a localized juvenile periodontitis patient, serotype b-specific rabbit antisera, serotype b bacterial sonic extract. (C) Serum from an adult periodontitis patient, serotype c-specific rabbit antisera, serotype c bacterial sonic extract.

Table 4 presents the relationship between *A. actinomycetemcomitans* serotypes and the fermentation of dextrin, maltose, mannitol, and xylose. These carbohydrates are variably fermented by *A. actinomycetemcomitans*, and they have been used in the biotyping of *A. actinomycetemcomitans* (26). *A. actinomycetemcomitans* serotype b strains fermented xylose, whereas serotype a strains did not ferment xylose. Serotype c strains were variable with respect to xylose fermentation, as were all three *A. actinomycetemcomitans* serotypes relative to the fermentation of the other three carbohydrates tested.

DISCUSSION

This study demonstrates that three antigenic groups exist among oral *A. actinomycetemcomitans*. These data were obtained by using Ouchterlony gel diffusion, indirect immunofluorescence, and whole and immunoabsorbed rabbit

TABLE 4. Relationship between *A. actinomycetemcomitans* serotype and carbohydrate fermentation

Serotype	Total no. of isolates	No. of isolates (%) fermenting:			
		Xylose	Mannitol	Dextrin	Maltose
a	97	0 (0)	86 (89)	49 (51)	69 (71)
b	139	139 (100)	31 (22)	26 (19)	39 (28)
c	36	6 (16)	15 (42)	9 (25)	30 (83)

antisera. These serological groups were designated serotypes in accordance with the *International Code of Nomenclature of Bacteria and Viruses* (9) and the serological nomenclature for the *Haemophilus* group of organisms. We designated the three serotypes a, b, and c in order of their identification.

Our findings agree with those of King and Tatum who, on the basis of capillary tube precipitation and whole rabbit antisera, also demonstrated three serological groups among 33 *A. actinomycetemcomitans* strains from various clinical infections (11). Although their serological groups showed no correlation with carbohydrate fermentation, our serotype a included only xylose-negative strains, and serotype b included only xylose-positive strains.

Taichman et al. (28) included a heat-labile toxin capable of lysing human polymorphonuclear leukocytes in vitro in the serotyping of *A. actinomycetemcomitans* and reported on four serotypes of *A. actinomycetemcomitans*. The serological categorization of certain strains of *A. actinomycetemcomitans* may be difficult under this scheme. *A. actinomycetemcomitans* ATCC 29523, for example, can vary with regard to leukotoxin production (28), which may reflect a variable presence of the leukotoxic antigen.

Consistent with previous studies is our finding that *A. actinomycetemcomitans* has a species-common antigen (11, 14), as well as an antigen shared with *H. aphrophilus* (11). In addition, Wetmore et al. (32) demonstrated common antigens among *A. suis*, *A. equuli*, and *A. lignieresii*, a finding with which we concur and which we have extended to include *A. actinomycetemcomitans*. In the identification of *Actinobacillus* sp., the use of common antigens can pose diagnostic difficulties. Pathak and Ristic (22) reported five human sputum isolates as *A. lignieresii* which were identified partly on the basis of common antigens in immunodiffusion assays; however, recent information suggests that their isolates may, in fact, have belonged to *Pasteurella ureae* (4). Whether some common antigens may be useful in the identification of genera or species of the *Haemophilus-Pasteurella-Actinobacillus* group of organisms is a matter for further study.

The primary ecological niche for *A. actinomycetemcomitans* is assumed to be the oral cavity and the upper respiratory tract. From these sites, *A. actinomycetemcomitans* may seed to various areas throughout the human body (2, 11, 21). Our finding that the type culture collection strains of *A. actinomycetemcomitans*, which have been isolated from medical infections in various parts of the body, share serotype antigens with oral *A. actinomycetemcomitans* suggests that these strains may, in fact, have originated in the oral cavity. In addition, in a patient with bacterial endocarditis, we have recently found biotypical and serological identity between *A. actinomycetemcomitans* strains isolated from the blood and from the oral cavity (C. S. Pierce, W. R. Bartholomew, J. J. Zambon, D. Amsterdam, and E. Neter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C42, p. 318). However, the possibility that a nonoral reservoir of *A. actinomycetemcomitans* also exists cannot be eliminated. If so, it is possible that nonoral *A. actinomycetemcomitans* may be antigenically distinct and comprise additional serotypes.

This study also indicates that specific *A. actinomycetemocomitans* serotypes and the corresponding serotype antigens may be more pathogenic in certain diseases. Although in healthy individuals, the approximately equal number of subjects infected with *A. actinomycetemcomitans* serotypes a and b indicates that neither serotype has an advantage in the initial colonization of periodontal sites, the increased proportions of serotype b in localized juvenile periodontitis suggest that serotype b strains may be particularly virulent in destroying periodontal tissues. Consistent with this observation is the finding that more *A. actinomycetemcomitans* serotype b strains produce leukotoxin than do serotypes a or c strains (J. J. Zambon, J. Slots, C. DeLuca, and R. J. Genco, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C41, p. 318). One may postulate a pathogenic mechanism for *A. actinomycetemcomitans* in localized juvenile periodontitis, in which *A. actinomycetemcomitans*, after the colonization of subgingival sites, may impair polymorphonuclear leukocytes by the effect of leukotoxin, which might then facilitate the invasion by *A. actinomycetemcomitans* of the gingival connective tissue (L. A. Christersson, B. Albini, J. Zambon, J. Slots, and R. J. Genco, J. Dent. Res. Special Issue A, Abstr. Am. Assoc. Dent. Res. 1983, no. 255). In addition, once *A. actinomycetemcomitans* has entered gingival sites, its capsular polysaccharide (8), which may, in fact, be the serotype-specific antigens, might inhibit phagocytosis by polymorphonuclear leukocytes and macrophages in a manner similar to that of the *H. influenzae* serotype b capsular polysaccharide (32). After invasion of the periodontal tissues, additional toxic products of *A. actinomycetemcomitans*, such as collagenase and lipopolysaccharide, could then destroy periodontal collagen fibers and bone.

A. actinomycetemcomitans serotypes a and c, on the other hand, may be especially important etiological agents in other diseases. Heinrich and Pulverer (6, 7) examined actinomycotic lesions and isolated 11 biochemically and serologically identical strains of *A. actinomycetemcomi-*

tans, which King and Tatum (11) determined to belong to their predominantly xylose-nonfermenting subgroup of *A. actinomycetemcomitans*. Most of these strains would probably belong to our serotypes a or c. *A. actinomycetemcomitans* serotype c, which is a rare periodontal isolate, has been isolated by us from both a lymphoma patient and a bacterial endocarditis patient (Table 3). Also, the type culture collection *A. actinomycetemcomitans* serotype c strains originate from vertebral and pulmonary infections.

Serotype selectivity for other organisms has also been reported in various nonoral diseases. An example which may be of interest for this study is that of *H. influenzae*. Of the six reported serotypes of *H. influenzae*, childhood meningitis is almost exclusively associated with serotype b, defined by the capsular polysaccharide (33).

In summary, this study has revealed the presence of three distinct surface antigens of *A. actinomycetemcomitans*. This information may be useful both in the identification of the organism in clinical specimens and in investigating antigens important in the pathogenesis of oral and nonoral infections caused by *A. actinomycetemcomitans*.

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