Distribution of Cross-reactive Antigens A and B in *Streptococcus mutans* and Other Oral Streptococci

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A collection of strains representing the various common species of oral streptococci was examined for the presence of antigens cross-reacting with antigens A and B from *Streptococcus mutans* serotype *c*. Antigens cross-reacting with A were found only in *S. mutans* serotype *b*. Antigens cross-reacting with B were found in *S. mutans* serotypes *a*, *c*, *d*, *e*, *f* and *g* and also in strains of *S. sanguis*. All antigens cross-reacting with B were precipitated by antiserum to human heart tissue.

INTRODUCTION

Recent advances in the taxonomy of the 'viridans' group of streptococci commonly found in the oral cavity have resulted in general agreement on the boundaries between the species *Streptococcus salivarius*, *S. mutans*, *S. milleri*, *S. sanguis* and *S. mitior* (also called *S. mitis*), though some differences of opinion remain concerning the relationship between the last two (Colman, 1976; Facklam, 1977; Hardie & Marsh, 1978). The taxonomic divisions are based primarily on physiological characteristics, supported in some cases by studies of cell wall composition or DNA homology (Colman & Williams, 1972; Coykendall & Munzenmaier, 1978). Studies on the serology of the viridans streptococci, however, have demonstrated extensive cross-reactions between species, though the cross-reacting antigens have not generally been identified (Colman, 1976; Colman & Williams, 1972; Facklam, 1977; Hardie & Bowden, 1976; Hardie & Marsh, 1978).

The antigenic structure of *S. mutans* is of particular interest because of the role played by this organism in the initiation of dental caries and the fact that vaccines based on *S. mutans* can protect experimental animals against this disease (Bowen, 1976). The polysaccharide antigens of *S. mutans*, on which the serotyping system is based, have been extensively studied (Linzer *et al.*, 1976), but little is yet known about the protein antigens of the species (Bratthall & Pettersson, 1976). Recent reports described the purification of two wall-associated protein antigens of *S. mutans* serotype *c*: these antigens, designated A and B, had molecular weights of 29000 and 190000, respectively (Russell, 1979*a, b*). Antigen B was of particular interest because it showed antigenic cross-reactivity with human heart tissue (Hughes, 1979; Hughes *et al.*, 1980; Russell, 1979*b*). Antibody responses to both antigens A and B were found in monkeys immunized against dental caries (Cohen *et al.*, 1979), though the role they play in protection has yet to be elucidated. The present communication describes an investigation into the distribution of these antigens in the various serotypes of *S. mutans* and in representatives of other streptococcal species.

METHODS

Organisms. The following strains of *Streptococcus mutans* were used: serotype *a* – AHT, OMZ61, NIDR 3720; serotype *b* – FA1, OMZ51, OMZ71; serotype *c* – Ingbritt, GS5, NCTC 10449; serotype *d* – OMZ176, OIH1, B13; serotype *e* – P4, NCTC 10923, At10; serotype *f* – SE11, 151, 4177; serotype *g* – K1, OMZ65, TH62. The sources of these strains were described previously (Russell, 1976) with the exception of strain AHT which was obtained from D. Bratthall and confirmed as type *a* (Russell, 1979*d*). Streptococci other than *S. mutans* were kindly provided by G. Colman of this laboratory. *Streptococcus sanguis* strains are listed in Table 2. Other strains used were *S. mitior* FW9, FW63 and FW75, *S. milleri* IS8, IS57, 7180, S198, FW702 and OG7331, and *S. salivarius* FW43 and BU104.

Media and culture conditions. Bacteria were grown in Todd–Hewitt broth (Oxoid) for 20 h at 37 °C.

Extraction methods. Bacteria from a culture grown for 20 h in 20 ml Todd–Hewitt broth were collected by centrifugation. For acid extraction, the pellet was heated with 50 µl 0.2 M-HCl at 100 °C for 10 min. For autoclave extraction, the pellet was heated with 50 µl 0.9 % (w/v) NaCl at 121 °C for 15 min. For extraction by mutanolysin, the pellet was suspended in 0.01 M-Tris/maleate/NaOH buffer (pH 7.0) containing mutanolysin M-1 enzyme (Yokogawa *et al.*, 1975; 200 µg ml⁻¹) and incubated for 3 h at 37 °C. The extracted slurries were used for immunodiffusion without further centrifugation. Mutanolysin was generously provided by M. Yokogawa, Dainippon Pharmaceutical Company, Japan.

Preparation of antisera. Typing sera were prepared in rabbits by a course of intravenous injections of heat-killed cells of *S. mutans* as described by Bratthall (1970). The production of antiserum to pure antigens A and B has been described (Russell, 1979*a, b*) and rabbit antiserum to human heart tissue was kindly provided by Dr M. Hughes, Wellcome Research Laboratories (Hughes, 1979; Hughes *et al.*, 1980).

Serological methods. Agglutination of broth cultures grown for 16 h was tested by mixing a drop of culture with 5 µl antiserum on a microscope slide and rocking it gently. Agglutination, when it occurred, was apparent within 5 min. Immunodiffusion was performed in 1 % (w/v) agarose in 0.05 M-Tris/HCl buffer (pH 7.5) on glass slides. Samples of 20 µl antigen preparation or antiserum were placed in 4 mm diameter wells 4 mm apart. Slides were dried, washed in saline and the immunoprecipitates were stained with Coomassie blue as described by Axelsen *et al.* (1973).

Preparations of antigens. Lipoteichoic acid and serotype polysaccharides were extracted from bacteria with hot aqueous phenol (Westphal & Jann, 1965). The antigens were then purified by gel filtration on a column of Sepharose 6B followed by ion-exchange chromatography on DEAE-Sephadex, essentially as described by Linzer *et al.* (1976). Lipoteichoic acid was prepared from strain Ingbritt, and serotype polysaccharides from AHT, FA1, Ingbritt and K1.

RESULTS

Antibodies in typing sera

Antigens A and B were purified from *S. mutans* strain Ingbritt (serotype *c*) and antigenically identical proteins were detected in strains of serotypes *e* and *f*; serotype *b* strains contained an antigen cross-reacting with A while strains of serotypes *d* and *g* contained antigens cross-reacting with B (Russell, 1979*a, b*). The occurrence of these antigens in more than one serotype suggested that antibodies to antigens A or B might be a cause of lack of specificity of typing sera. A collection of sera prepared in this laboratory for serotyping purposes was therefore examined by immunodiffusion for the presence of antibodies to a number of defined antigens. All the typing sera were prepared by intravenous injection of rabbits with heat-killed whole cells and 10 out of 12 sera had the desired anti-polysaccharide antibodies, though four were non-specific due to the presence of antibodies to lipoteichoic acid (Table 1). Precipitin lines of polysaccharide or lipoteichoic acid appeared sooner and were stronger than those containing protein antigens when slides were viewed on a dark-ground lightbox, but slides stained with Coomassie blue clearly revealed the presence of precipitates to antigen B in six of the sera and to antigen A in three sera (Table 1). Figure 1 shows the reactions of antiserum against strain GS5 with extracts from representatives from all seven serotypes.

Fig. 1

Fig. 2

Fig. 1. Immunodiffusion of antiserum raised against whole cells of strain GS5 (centre well) and autoclave extracts of representatives of the different *S. mutans* serotypes. The line close to the antiserum well is due to antibody to serotype *c* polysaccharide, while the other lines are due to antibody to antigen B.

Fig. 2. Immunodiffusion of antisera raised against SDS-extracted cells of different *S. mutans* serotypes and pure antigen B from strain Ingbritt. 1, anti-FA1 (*b*); 2, anti-OMZ61 (*a*); 3, anti-Ingbritt (*c*); 4, anti-K1 (*g*).

Table 1. *Antibodies present in sera prepared for serotyping S. mutans*

Each serum came from a different rabbit immunized with heat-killed *S. mutans*. Sera were tested by two-dimensional immunodiffusion against four purified antigens. The polysaccharide antigen used was that prepared from a strain of the same serotype as the immunizing strain.

Immunizing strain	Serotype	Antigen*			
		PS	LTA	A	B
AHT	<i>a</i>	+	—	—	—
OMZ61	<i>a</i>	—	—	—	+
FA1	<i>b</i>	+	+	+	—
FA1	<i>b</i>	+	+	+	—
FA1	<i>b</i>	+	—	—	—
GS5	<i>c</i>	+	—	—	+
Ingbritt	<i>c</i>	+	—	+	+
Ingbritt	<i>c</i>	—	—	—	+
Ingbritt	<i>c</i>	+	+	—	+
K1	<i>g</i>	+	+	—	+
K1	<i>g</i>	+	—	—	—
K1	<i>g</i>	+	—	—	—

* PS, Serotype-specific polysaccharide; LTA, lipoteichoic acid.

Presence of antigen B in serotype a

Evidence from the typing sera indicated that antiserum raised against the serotype *a* strain OMZ61 reacted with antigen B, although no cross-reacting antigen had previously been detected in culture filtrates, autoclave or acid extracts of strains of this serotype (Russell, 1979*b*). Antigen B from strain Ingbritt was originally characterized as an antigen which remained on the bacteria even after boiling in sodium dodecyl sulphate (SDS) (Russell, 1979*b*). That an analogous antigen existed in serotype *a* was demonstrated by using SDS-extracted cells of OMZ61 to immunize a rabbit. The resultant antiserum precipitated antigen B (Fig. 2). The same approach failed to detect any such antigen in strain FA1 (serotype *b*). Further evidence for the presence of antigen B in serotype *a* strains was provided by the observation of the slide agglutination of serotype *a* strains by antiserum raised against pure antigen B (Table 2).

Table 2. *Detection of antigens reacting with antiserum to antigen B*

Strain		Detection method		
		Agglutination	Autoclave extract	Mutanolysin extract
<i>S. mutans</i> *				
Serotype	<i>a</i>	+	—	+
	<i>b</i>	—	—	—
	<i>c</i>	+	+	+
	<i>d</i>	x †	+	+
	<i>e</i>	+	+	+
	<i>f</i>	+	+	+
	<i>g</i>	x †	+	+
<i>S. sanguis</i>				
Type I	MWK	—	—	+
	NCTC 7865	+	—	+
	NCTC 7869	—	—	+
	NCTC 10231	—	—	—
Type II	S159	—	+	+
	Bu174	—	+	+
	NCTC 7864	—	+	+

* Three representatives of each serotype were examined; no variations were found within a serotype.

† Autoagglutination in the absence of serum.

Extraction of antigen B with mutanolysin

The above results indicated that strains of serotype *a* possessed an antigen resembling antigen B, but that it was not as readily released as the corresponding antigen from other serotypes. An alternative method for release of antigen was therefore sought. Mutanolysin is an enzyme from *Streptomyces globisporus* which has been reported to lyse all serotypes of *S. mutans* and other streptococci (Hamada *et al.*, 1978). We confirmed these observations and found that digestion with mutanolysin efficiently released antigen B from all strains known to contain it, including those of serotype *a* (Table 2). No cross-reacting antigen was released from any of the three serotype *b* strains.

Antigens in other streptococci

The methods found suitable for detecting antigen in the *S. mutans* strains were applied to a collection of representative strains of the other streptococci commonly found in the mouth. An antigen cross-reacting with B was detected in six out of seven strains of *S. sanguis* (Table 2). No such antigen was found in the strains of *S. mitior*, *S. milleri* or *S. salivarius*. The presence of an antigen cross-reacting with antigen A from strain Ingbritt was previously reported in serotype *b* strains (Russell, 1979*a*). The present survey confirmed this finding, but failed to detect a cross-reacting antigen in any of the other strains examined.

Heart cross-reactivity

Antigen B from *S. mutans* strain Ingbritt has been shown to be precipitated by rabbit antiserum raised against human heart tissue (Hughes, 1979; Hughes *et al.*, 1980; Russell, 1979*b*). In immunodiffusion the same anti-heart serum reacted with all those extracts of *S. mutans* and *S. sanguis* which contained antigen cross-reacting with antigen B.

DISCUSSION

The antigens involved in cross-reactivity between oral streptococci have not been identified. Lipoteichoic acid and dextran are believed to be responsible for some cross-

reactions (Bratthall, 1969; Chorpenning *et al.*, 1975), and Hardie & Bowden (1976) presented preliminary evidence that a protein antigen was common to *S. mutans* and *S. sanguis*. I recently characterized antigen B of *S. mutans* strain Ingbritt as a wall-associated protein with an apparent molecular weight of 190000 and the present report shows that six of the seven serotypes of *S. mutans* contain this or a closely similar antigen, as do strains of *S. sanguis*. It is perhaps surprising that *S. mutans* serotype *b* strains lacked the antigen as serotypes *b* and *c* are closely related genetically (Coykendall, 1974) and have already been shown to have antigenically cross-reacting glucosyltransferase (Russell, 1979*c*) and antigen A (Russell, 1979*a*). The possibility remains that a cross-reacting antigen might be discovered if a wider range of growth conditions and extraction methods were introduced.

Cross-reacting antigen B was detected in strains of both *S. sanguis* type I and *S. sanguis* type II but not in strains of *S. mitior*. Some authors have suggested *S. sanguis* type II strains should be classified as *S. mitior* from which they differ only in the ability to synthesize a water-soluble glucan (Coykendall & Munzenmaier, 1978), and while this might suggest that the antigen common to all the *S. sanguis* strains is glucosyltransferase, clear evidence has been presented that glucosyltransferase and antigen B from *S. mutans* strain Ingbritt are distinct entities (Russell, 1979*b*).

The most widely employed technique for producing sera for typing *S. mutans* involves intravenous injection of whole bacteria into rabbits and it is generally recognized that one or more absorption steps are needed to produce serotype-specific sera. The results presented in Table 1 indicate that antigens A and B may frequently be the causes of the non-specificity of sera.

A knowledge of antigenic determinants which may be shared between species is essential in any studies in which attempts are made to assess levels of antibody to a particular organism. For instance, numerous attempts have been made to correlate levels of antibody to *S. mutans* with caries experience, but it is now clear that if whole bacteria are used in immunoassay procedures not only specific antibody will be detected, but also antibody to such non-specific antigens as lipoteichoic acid (Decker *et al.*, 1972; Markham *et al.*, 1973) and antigen B.

Characterization of the antigens involved in heart cross-reactivity and their distribution is also needed for appreciation of the hazards involved in the development of streptococcal vaccines. Furthermore, a knowledge of the antigenic relationships of different oral streptococci may help provide information on the mechanism by which immunity to dental caries is obtained, as it is clear that immunization with *S. mutans* may induce antibodies capable of reacting with other species.

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