Immunochemical Relationship between Streptococcus MG, F III and *Streptococcus salivarius*

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SUMMARY

The identity of the polysaccharide antigens of *Streptococcus* MG and streptococci with group antigen F and type antigen III is shown by precipitation reactions with formamide extracts, cross-absorption tests, sugar composition and by the inhibition of the quantitative precipitation reaction. On this basis *Streptococcus* MG can be classified as an F III strain.

The relationship between *Streptococcus* MG and *Streptococcus salivarius* type I is explained by their common reactions with anti-III serum. Inhibition of the quantitative precipitations with simple sugars suggest that the determinant groups of the type III and the 'salivarius' antigen are not quite identical, although both antigens contain a β -glucosidic and a galactosidic group.

A second unrelated antigen has been found in some 'salivarius' strains alone (represented by strain 51) or together with type III antigen. The quantitative precipitation of the strain 51/anti-strain 51 system was only slightly inhibited by rhamnose.

The qualitative composition of the sugar components of the formamide extracts of *Streptococcus* MG, F III, both 'salivarius' types of O III are compared.

INTRODUCTION

Mirick, Thomas, Curnen & Horsfall (1944) isolated from the lungs of a fatal case of primary atypical pneumonia a streptococcus strain which was first designated *Streptococcus* 344, later on *Streptococcus* MG. These authors described *Streptococcus* MG as a separate serological entity (Mirick *et al.* 1944 *a*) only related to *Streptococcus salivarius* type I (Mirick *et al.* 1944 *b*) as described by Sherman, Niven & Smiley (1943). *Streptococcus* MG gives capsular swelling and agglutination with homologous serum. Precipitation reactions can be done with various kinds of extracts of these streptococci.

Streptococcus MG (NCTC 8037/50) is used in several laboratories as a standard strain in testing for MG antibodies.

Many indifferent (no α - or β -haemolysis) and haemolytic streptococci isolated from dental root canals contain the group antigen F and one of the type antigens I-V observed in this group (Ottens & Winkler, 1962). The serological identification of these strains was based on the precipitin reaction with the soluble polysaccharide antigens obtained by hot formamide extraction of whole cocci. Data about the chemical constitution of these group and type antigens and about the structure of

the determinant groups of these antigens were described by Willers, Michel, Sijsma & Winkler (1964).

In the present paper data are given for the identity of the carbohydrate antigens of *Streptococcus* MG (NCTC 8037/50) and an F strain with type antigen III. Many *Streptococcus salivarius* strains also contain an antigen reacting with anti-III serum. The serological relationships between these strains and the chemical composition of their antigens are described.

METHODS

Strains. *Streptococcus* MG (NCTC 8037/50), *Streptococcus salivarius* (NCTC 8606) and 16 strains provisionally classified as MG strains were obtained from Dr C. E. de Moor (National Institute of Public Health, Utrecht, The Netherlands). Other streptococci also provisionally classified as MG strains were obtained from Professor Dr M. Seeleman (Institute of Dairying Hygiene, Kiel, Germany). Forty-two *S. salivarius* strains were isolated from dental plaque material (*materia alba*).

The qualitative chemical analysis was performed by the method of Michel & Gooder (1962).

The formamide extraction of the bacteria, the purification of the crude formamide extracts, the isolation of polysaccharides from the medium, the quantitative precipitin inhibition technique and the preparation of sera against group F and against the types I up to V have been described earlier (Willers *et al.* 1964). Anti-MG serum was obtained from Dr C. E. de Moor. All other anti-group sera were from Burroughs Wellcome. Anti-A variant serum was a generous gift of Mr W. R. Maxted.

Absorption of sera. Heat-killed streptococci were washed twice with saline and finally centrifuged at 36,000 *g*. Two volumes of serum were mixed with the sedimented bacteria, incubated during 1 hr at 37° and centrifuged at 36,000 *g*.

RESULTS

Serology. Formamide extracts of *Streptococcus* MG (NCTC 8037/50) gave positive reactions with anti-F and anti-III sera. Polysaccharides isolated from the medium reacted with anti-III serum only.

Anti-MG serum reacted with formamide extracts of the homologous strain and with strains containing the type III antigen.

The cross-absorption tests in Table 1 show that the anti-MG serum used is an anti-III serum. This is in accordance with our general experience that streptococci carrying group and type antigens often give rise to antibodies against the type antigen only. From these data it can be concluded that *Streptococcus* MG (NCTC 8037/50) is serologically identical with an F III strain.

Sixteen strains of the National Institute of Public Health (Utrecht) and 5 strains of the Institute for Dairying Hygiene (Kiel) which were classified as *Streptococcus* MG were tested in the capsular swelling test with anti-MG and anti-III sera. With formamide extracts of these strains capillary precipitin tests were made with all Lancefield group sera and with the type sera of group F. The correlation between capsular swelling and the precipitin reaction (Table 2) with anti-III serum is clear. Some strains gave a precipitin reaction with anti-F serum or with an anti-L serum (Burroughs Wellcome). In five strains no group antigen was detected. These

strains were designated as 0 III (zero III). The strains which gave no positive capsular swelling reaction are probably not true MG strains.

Three out of the five 0 III strains in the first group gave big slimy colonies on 5% sucrose agar. They were therefore classified as *Streptococcus salivarius*.

Table 1. *Absorptions of anti-MG serum with different strains of bacteria*

Anti-MG serum absorbed with cells of	Formamide extract of		
	C III	F III	MG
C III	—	—	—
F III	—	—	—
MG	—	—	—
F	+	+	+
None	+	+	+

Table 2. *Capsular swelling tests and precipitin reactions of strains classified as Streptococcus MG*

No. of strains	Capsular swelling tests with antiserum against		Precipitation reactions with antiserum against		
	Type III	MG	Group L	Group F	Type III
8	+	+	—	+	+
3	+	+	+	—	+
5	+	+	—	—	+
2	+	+	—	+	+
1	+	+	—	—	+
2	—	—	—	—	—

The first group of strains was obtained from the National Institute for Public Health, Utrecht, Netherlands. The second group was obtained from the Institute for Dairying Hygiene, Kiel, Germany.

Streptococcus salivarius (NCTC 8606) and 25 out of 42 freshly isolated *S. salivarius* strains gave positive precipitin reactions with an anti-III serum. Antisera against *salivarius* NCTC 8606 and against one of the strains without type III antigen (strain 51) were prepared. The reactions of the formamide extracts of both 'salivarius' strains and an F III strain, with their respective antisera, are given in Table 3. It is clear that strain 51 contains an antigen different from type III antigen, whereas both antigens are present in strain 8606. These data were confirmed with cross absorption tests.

Formamide extracts of 42 'salivarius' strains were tested with anti-III, anti-8606 and anti-51 sera (Table 4). All strains react with anti-8606 serum; 5 strains contain the III antigen, 12 strains the 51 antigen and 25 strains both antigens, like strain 8606.

Chemical composition of carbohydrates in formamide extracts. Qualitative chemical analysis of various strains (Table 5) showed that in both F III and MG (NCTC 8087/50) galactosamine which represents the F antigen (Michel & Willers 1964) was the main hexosamine.

Glucosamine was present in low amounts in F III but not in MG. Glucose, galactose and rhamnose were found in large amounts in F III and MG strains.

The right-hand part of Table 5 gives a comparison of the results of the qualitative composition of *Streptococcus salivarius* (NCTC 8606), *S. salivarius* 51 and an O III strain. All contain glucosamine, glucose, galactose and rhamnose; *S. salivarius* 51 contains in addition mannose.

Table 3. *Cross-reactions and cross-absorptions of anti-salivarius NCTC 8606, anti-salivarius 51, and anti-III sera*

Serum	Absorbed with	Formamide extract of		
		Sal 8606	F III	Sal 51
Anti-sal 8606	.	+++	+++	++
Anti-sal 51	.	++	—	++
Anti-III	.	+++	+++	—
Anti-sal 8606	Sal 51	+++	+++	—
Anti-sal 8606	F III	±	—	++
Anti-sal 51	Sal 8606	—	—	—
Anti-III	Sal 8606	—	—	—

+++ = precipitation immediately positive; ++ = precipitation positive in 30 sec.; + = precipitation positive in 2 min.; ± = weak precipitation reaction within 5 min.; — = no precipitation within 5 min.

Table 4. *Reactions of salivarius strains with anti-salivarius and anti-III sera*

Number of isolated strains	Anti-8606 serum	Anti-51 serum	Anti-III serum
25	+	+	+
12	+	+	—
5	+	—	+

Table 5. *Qualitative sugar composition of formamide extracts of Streptococcus MG, F III, O III and Streptococcus salivarius NCTC 8606 and strain 51*

	MG NCTC 8037	F III	O III	Salivarius NCTC 8606	Salivarius 51
Galactosamine	+	+++	—	—	—
Glucosamine	—	+	+	+	+
Galactose	++	++	+++	+++	++
Glucose	+++	+++	+++	+++	+++
Rhamnose	+++	+++	+++	+++	+++
Mannose	—	—	—	—	+
N-Acetylhexosamine	±	++	+	++	++

Inhibition of the quantitative precipitation. The results of the inhibition reactions by simple sugars of the III/anti-III and MG/anti-MG reaction are compared (Table 6). Only the sugars giving at least 30 % inhibition are shown; for other sugars tested see Willers *et al.* (1964). In both systems the same sugars gave a measurable amount of inhibition at the sugar levels used. These results suggest that the determinant groups of the type III antigen have many properties in common with those of an antigen of *Streptococcus* MG. As mentioned before *Streptococcus* MG only gives rise to antibodies against the type III antigen. Therefore the inhibitions

of the precipitin reaction of MG antigen and anti-F serum were studied. 50% inhibition was reached by $8\mu\text{M}$ -cellobiose and by $0.01\mu\text{M}$ -3-O- β -D-glucopyranosyl-N-acetyl-D-galactosamine. As the latter is a disaccharide of the determinant group of the F antigen (Michel *et al.* 1964) the results of these inhibitions give further evidence for the presence of the F antigen in the formamide extracts of Streptococcus MG.

Table 6. *Inhibition by simple sugars of precipitin reactions of the type III and the MG systems*

	III/anti-III	MG/anti-MG
Glucose	90*	42
β -Methylglucose	46	32
Cellobiose	34	26
Gentiobiose	18	18
α -Methylglucose	41†	55†
Galactose	90	33
Melibiose	60	22†
Glucosamine	.	37
Galactosamine	.	48†

* 50% inhibition value expressed in μM -sugar.

† 30% value, where the 50% value was not reached.

Table 7. *Inhibition by simple sugars of the precipitin reactions of the type III and both salivarius systems, the cross reacting systems of type III and salivarius NCTC 8606 and the system salivarius 8606 antigen/anti-salivarius 8606 adsorbed with salivarius 51 bacteria*

	III/ anti-III	8606/ anti-III	III anti-8606	8606/ anti-8606	8606/ anti-8606/ absorbed with 51 bacteria	51/ anti-51
Glucose	90*	30	88†	44	59	—
β -Methylglucoside	46	14	14	7	5	—
Cellobiose	34	8	48†	30	55	—
Gentiobiose	18	6	16	8	8	—
α -Methylglucoside	41†	82	110†	82	96	110†
Galactose	90	15	59†	30	44	103†
Melibiose	60	7	44†	37	60	60†
Rhamnose	—	114†	—	137†	130†	113
Glucosamine	—	100	40†	62	87	100
Galactosamine	—	50†	74†	100	99	62†

* 50% inhibition value expressed in μM -sugar.

† 30% value, where the 50% value was not reached.

The inhibition reactions of the different 'salivarius' systems, the III system and an absorbed anti-salivarius 8608/salivarius 8606 system were compared with each other (Table 7). Except for the 'salivarius' 51 system, the same sugars as in the III/anti-III system were active. In the 'salivarius' 51 system rhamnose gave a weak inhibition reaction. Because anti-A variant serum is directed against rhamnose endgroups (McCarty 1956) this serum was tested against a formamide extract of *S. salivarius* 51; it was inactive.

DISCUSSION

Soon after its discovery in 1948 *Streptococcus* MG was serologically examined and classified as a separate genus, which was only related to *Streptococcus salivarius* type I as described by Sherman *et al.* (1948). The precipitin reactions with formamide extracts, the cross-absorption tests and the inhibition of the quantitative precipitin reaction suggest that *Streptococcus* MG contains the same polysaccharide antigens as *Streptococcus* F III and can be classified as F III.

All strains containing the III antigen will react with an anti-MG serum. F III, L III and O III strains were positive (Table 2) and as the III antigen is also found in combination with the group antigen C (Ottens & Winkler, 1962), these c III strains will also give positive reactions with anti-MG sera. Part of the strains classified as O III proved to be *Streptococcus salivarius*. The relationship between *Streptococcus* MG and *Streptococcus salivarius* type I of Sherman *et al.* (1948) as found by Mirick *et al.* (1944 *b*) can be explained by the reactions of both with anti-III serum. A second unrelated 'salivarius' antigen (strain 51) was found. The relationship between strain 51 antigen and the 'salivarius' type II antigen of Sherman could not be investigated, as the latter strain was not available.

Qualitative chemical analysis and the inhibition of the precipitin reaction gave further confirmatory information about the identity of *Streptococcus* MG and F III strains.

Qualitative of chemical analysis of the hydrolysates of the formamide extracts *Streptococcus salivarius* and O III strains showed the same sugar composition. Strain 51 however contained in addition mannose. The difference between the 'salivarius' and O III strains on the one hand and MG and F III on the other is shown by the difference in the hexosamines found. In the first group of strains glucosamine is found and galactosamine is lacking, whereas in the second group galactosamine is the main hexosamine. As has been shown earlier (Willers *et al.* 1964) galactosamine is the main hexosamine in the group F antigen. The inhibition of the precipitin reactions of the 'salivarius' and III systems (Table 7) are very similar. In both a β -glucosidic and a galactosidic group seem to act as a determinant of the antigens. Whenever anti-III serum is used cellobiose gives a better inhibition than β -methylglucoside and almost equals that given by gentiobiose. When, however, anti-salivarius 8606 serum is used the inhibiting capacity of β -methylglucoside and gentiobiose are the same and exceed that of celliobiose. From these differences it can be seen that anti-salivarius 8606 serum contains antibodies with combining sites that fit better with 1 $\rightarrow$ 6- β -glucosidic linkage than with a 1 $\rightarrow$ 4- β -glucosidic linkage. Although the type III and salivarius 8606 systems give strong cross reactions, these results suggest that the determinant groups of the antigens are not completely identical. A more detailed study with oligosaccharides obtained by partial hydrolysis of the antigens will be necessary to study possible differences between the determinant groups of the type III and salivarius 8606 antigens.

The inhibition pattern of strain 51 indicates a different antigenic structure. Only rhamnose gives a slight inhibition, a reaction found in the A variant (McCarty, 1956), C variant (Krause & McCarty, 1963) and group G (Curtis & Krause, 1964) systems.

No relationship between the variant A system and the 'salivarius' type 51 system was found.

The inhibition given by the hexosamines in several systems, in which the *N*-acetyl hexosamines were inactive is not quite clear. Good inhibitions given by arginine and lysine in the same systems suggest that anti-protein reactions are involved here.

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