

STUDIES ON THE ANTIGENIC COMPOSITION OF GROUP A
HEMOLYTIC STREPTOCOCCI

II. THE OCCURRENCE OF STRAINS IN A GIVEN TYPE CONTAINING M BUT NO
T ANTIGEN*†

BY REBECCA C. LANCEFIELD, PH.D.,
AND WALTER A. STEWART, § M.D.
Lieutenant (j.g.), Medical Corps, United States Naval Reserve

(From the Hospital of The Rockefeller Institute for Medical Research)

(Received for publication, September 29, 1943)

Previous studies on the antigenic composition of group A hemolytic streptococci have shown that the majority of matt variants contain at least two antigens related to type-specificity and designated as M and T (1). In a preceding paper, a new method of separating these two antigens was described (2). It was found that short exposure of group A streptococci to the action of proteolytic enzymes destroyed the M substance but left the T substance apparently unaltered. In most instances, therefore, immunization of rabbits with enzyme-treated streptococci led to the production of T antibodies. In the case of certain strains, however, the injection of suspensions so treated failed to induce the formation of T antibodies. The simplest explanation of this finding was that the T antigen characteristic of the type was lacking in these particular strains. At the present time, 28 strains apparently lacking in T antigen have been observed in eight different types. Evidence concerning their antigenic composition is presented in this paper.

Materials and Methods

The sources of the strains studied in detail are given in Table I. Other strains used for comparison, as well as the methods employed, are the same as those described in the preceding paper (2).

EXPERIMENTAL

A. Evidence That Certain Matt Strains Are Devoid of T Antigen.—

*1. Inagglutinability in Anti-T Serum.—*A member of type 14, designated as

* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

† The Bureau of Medicine and Surgery, Navy Department, does not necessarily undertake to endorse views or opinions which are expressed in this paper.

§ Member of the United States Navy Research Unit at the Hospital of The Rockefeller Institute for Medical Research.

strain C 630, is used as an example. It was found that, although this culture agglutinated in antisera containing the usual M and T agglutinins, it failed to react in anti-T sera which lacked M antibodies.

Special antisera were prepared to investigate this phenomenon. Rabbits were immunized with suspensions of heat-killed cultures of another strain of type 14 (S 23 matt), known to contain both M and T antigens (1). The serum thus prepared was divided into two lots: One was absorbed with a strain of a heterologous type to remove non-type-specific antibodies; the other with a glossy variant of the homologous strain to remove the anti-T agglutinins. An anti-T serum was made by immunizing rabbits with a trypsin-treated suspension of the same strain (S 23 matt), and then absorbed to remove non-type-specific antibodies. The agglutination reactions of strain C 630 in these anti-

TABLE I
Chief Strains Studied Which Contain M but No T Antigen

Type	Strain	Original source and designation
14	C 630	Uncertain
17	D 205	Schwentker, strain 6043
19	T 19	Griffith, " S.F. 73/4
24*	C 98	Rockefeller Hospital, strain 22 RS 72
26	C 179	" " " 11 RS 50
28	T 28	Griffith, strain Small
29	J 17 B	Coburn, " R 5
30	D 24	Griffith, " Quinn

* This type was previously designated as "provisional type 45" in this laboratory.

sera were compared with those of the matt and glossy variants of strain S 23, as shown in Table II.

Strain C 630 failed to react in serum which contained only anti-T agglutinins, although it agglutinated in any serum in which M antibodies for type 14 were present. On the other hand, strain S 23 matt, known to contain both M and T antigens, agglutinated in anti-M and anti-T sera; and strain S 23 glossy, known to contain little or no M antigen, reacted only in anti-T serum. These agglutination reactions, therefore, indicated that strain C 630 contained no T antigen.

2. Failure of Strain C 630 to Induce the Formation of T Antibodies When Injected into Rabbits.—Two rabbits were immunized with heat-killed suspensions of strain C 630, and two more with the same culture after exposure to chymotrypsin. The animals injected with the heat-killed suspensions produced the usual type-specific precipitins, agglutinins, and protective antibodies (2); but neither these rabbits nor those immunized with the enzyme-treated cultures gave any evidence of the production of T agglutinins.

Agglutination reactions, performed with unabsorbed sera and with sera

absorbed in different ways, are presented in Table III and show that, although strain C 630 contained M antigen, a T antigen similar to that found in most type 14 strains was lacking. In our experience T agglutinins are usually formed rapidly and in high titer, especially when enzyme-treated suspensions are used for immunization. It seemed likely, therefore, that the failure to obtain these antibodies was due to the absence of T antigen from strain C 630.

TABLE II
Inagglutinability of Strain C 630 in Anti-T Serum

Type 14 antisera	Strains used for agglutination reactions		
	C 630 matt (contains M antigen)	S 23 matt (contains M and T antigens)	S 23 glossy (contains T antigen)
A. <i>Serum from rabbit R57-44</i> immunized with trypsin-treated suspension of strain S 23 matt was absorbed with strain of heterologous type (after absorption con- tains T antibodies)	—	++*	++++
B. <i>Serum from rabbit R53-38</i> immunized with untreated suspension of strain S 23 matt was absorbed with strain of heterologous type (after absorption contains M and T antibodies)	++++	++++	++++
C. <i>Serum from rabbit R53-38</i> absorbed with glossy variant of homologous strain (S 23 glossy) (after absorption contains M antibodies)	++++	++++	—

Readings are a summary of the results obtained with a series of serum dilutions from 1:20 to 1:2560, recorded on a ± to ++++ scale.

— indicates negative reaction.

The techniques of absorbing the antisera and performing the agglutination tests are those previously described (1, 2).

*Some blocking of T agglutination was observed with this matt virulent strain. Cf. Table V.

3. *Failure of Strain C 630 to Absorb T Antibodies.*—Two type 14 antisera, one containing M and T antibodies and the other containing only T agglutinins, were absorbed with strain C 630 as well as with matt and glossy variants of another strain of type 14 (strain S 23) and with streptococci of a heterologous type. As shown in Table IV, absorption of both sera with strain C 630 failed to remove T agglutinins, although these antibodies were eliminated by absorption with either variant of strain S 23. On the other hand, anti-M agglutinins were removed by absorbing with strain C 630. These findings corroborated

those of the preceding experiments in indicating that strain C 630 lacked T antigen.

B. The Blocking of T Agglutination of Matt Strains Known to Contain T Antigen.—As demonstrated above, certain streptococci occur which, since they lack T antigen do not agglutinate in anti-T serum. Other streptococci have been encountered which resemble these strains in failing to agglutinate in anti-T serum, but differ in possessing demonstrable T antigen. The agglutina-

TABLE III
Failure of Strain C 630 to Induce the Formation of T Antibodies in Rabbits

Type 14 antisera	Strains used for agglutination reactions		
	S 23 glossy (contains T antigen)	S 23 matt (contains M and T antigens)	C 630 matt (contains M antigen)
A. Serum from rabbit R51-55			
immunized with trypsin-treated suspension of strain C 630 (contains no M or T antibodies)			
1. Unabsorbed.....	±	—	—
2. Absorbed with strain of heterologous type.....	—	—	—
B. Serum from rabbit R50-43			
immunized with untreated culture of strain C 630 (contains M antibodies)			
1. Unabsorbed.....	+	++++	++++
2. Absorbed with:			
(a) Strain C 630 matt.....	—	—	—
(b) " S 23 ".....	—	—	—
(c) " S 23 glossy.....	—	++++	++++
(d) " of heterologous type.....	—	++++	++++

See footnotes, Table II.

tion reactions of a matt strain of type 6 (S 43) are shown in Table V to illustrate the blocking of T agglutination, although it has been proved that this strain contains T antigen. Three variants were studied: a matt virulent, a matt avirulent, and a glossy variant. The matt virulent strain, which had been previously shown to agglutinate in anti-M serum, failed to agglutinate in anti-T serum, although the anti-T serum agglutinated the other two variants.

By subjecting the matt virulent culture to slightly unfavorable conditions, *e.g.* aging in the ice box, slight exposure to trypsin, or growing at room temperature, the strain usually became agglutinable in anti-T serum. The results of such an experiment with the type 6 strain, S 43, are recorded in Table VI.

In order to determine whether this matt virulent strain contained T antigen,

TABLE IV
Failure of Strain C 630 to Absorb Antibodies of Homologous Type

Type 14 antisera	Strains used for agglutination reactions		
	S 23 glossy (contains T antigen)	S 23 matt (contains M and T antigens)	C 630 matt (contains M antigen)
A. Serum from rabbit R53-38			
immunized with untreated suspension of strain S 23 matt (contains M and T antibodies)			
1. Unabsorbed	++++	++++	++++
2. Absorbed with:			
(a) Strain C 630	++++	++*	—
(b) “ S 23 matt	—	—	—
(c) “ S 23 glossy	—	++++	++++
(d) “ of heterologous type (strain S 43, type 6)	++++	++++	++++
B. Serum from rabbit R57-44			
immunized with trypsin-treated suspension of strain S 23 matt (contains T antibodies)			
1. Unabsorbed	++++	++	—
2. Absorbed with:			
(a) Strain C 630	++++	++	—
(b) “ S 23 matt	—	—	—
(c) “ S 23 glossy	—	—	—
(d) “ of heterologous type (strain S 43, type 6)	++++	++	—

* Some blocking of T agglutination was observed with this matt virulent strain. Cf. Table V.

TABLE V
Blocking of T Agglutination by M Substance in the Bacterial Cell
Agglutination Reactions

Cultures used for agglutination reaction	Rabbit R51-41 immunized with enzyme-treated culture, strain S 43, type 6 (contains T agglutinins)			
Variants of strain S 43, type 6				
1. Matt virulent	—	—	—	—
2. “ avirulent	+++	+++	++	+
3. Glossy	++++	++++	+++	++

Final serum dilutions 1:40, 160, 640, 2560.

two sets of rabbits were immunized, respectively, with enzyme-treated and with untreated cultures (Table VII). In both instances, anti-T agglutinins developed, as shown by the fact that these sera agglutinated a homologous glossy

TABLE VI
Effect of Various Modes of Treatment on the Agglutinability of Cultures

Type 6 matt virulent culture used for agglutination reactions	Rabbits immunized with type 6							
	Untreated culture (serum R51-38 contains M and T antibodies)				Enzyme-treated culture (serum R51-41 contains T antibodies)			
A. Grown at 37°C.								
1. 16 hr. culture.....	++++	++++	++++	+	-	-	-	-
2. Aged 8 days (ice box).....	+++	++++	++++	++	++	+++	+++	+
3. Trypsin - treated 20 min.....	+++	++	+	-	+++	+++	++	-
4. Grown in presence of trypsin.....	++	++	++	-	++	++	±	-
B. Grown at 20°C.								
16 hr. culture.....	++++	++++	++	-	+++	++	+	-

See footnotes, Table II.

A culture (strain S 43, type 6) grown at 37°C. was used for agglutination reactions (1) immediately, (2) after storage in the ice box for 8 days, (3) after exposure to 1 per cent trypsin (Fairchild's) for 20 minutes, three successive washings with physiological salt solution, and final suspension in broth. Another culture (4) was grown in broth to which trypsin in a final concentration of 0.05 per cent was added, and still another (B) was grown at 20°C.

TABLE VII
Immunization and Absorption Experiments Showing That the Type 6 Matt Virulent Strain, S 43, Contains T Antigen

Immune rabbit sera type 6	Type 6 variants (of strain S 43) used in agglutination reactions	
	Glossy (contains T antigen)	Matt virulent (contains M and T antigens)
A. Rabbit R51-41 immunized with enzyme-treated matt virulent culture, strain S 43, type 6 (contains T antibodies).		
1. Unabsorbed.....	++++	-
2. Absorbed with type 6 matt virulent strain.....	-	-
3. " " " 6 glossy strain.....	-	-
4. " " strain of heterologous type.....	++++	-
B. Rabbit R51-38 immunized with untreated matt virulent culture, strain S 43, type 6 (contains M and T antibodies)		
1. Unabsorbed.....	++++	++++
2. Absorbed with type 6 matt virulent strain.....	-	-
3. " " " 6 glossy strain.....	-	++++
4. " " strain of heterologous type.....	++++	++++

See footnotes, Table II.

TABLE VIII
Analysis of Antigenic Composition of Type 14 Strains with Respect to M and T Antigens

Strains tested and their antigenic composition	Agglutination reactions*										Precipitin reactions† Anti-M serum	
	Anti-T serum					Anti-M serum						
<i>M and T antigens</i>												
1. S 23 matt.	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2. 23 RS 84.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3. S 65.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
4. S 39.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5. 277 HP.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	±
<i>T antigen</i>												
1. S 23 glossy.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
2. 35 RS 84.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
<i>M antigen</i>												
1. C 630.	-	-	-	-	-	-	-	-	-	-	-	+++
2. C 79.	-	-	-	-	-	-	-	-	-	-	-	+++

* Final serum dilution ranged from 1:20 through 1:640.

† Precipitin reactions were performed in dilutions as previously described.

variant. Furthermore, it was found that when anti-T sera were absorbed with the matt virulent strain, T agglutinins were removed, although more absorptions were required than when matt avirulent or glossy variants were used.

Seventeen other M-containing type 6 strains were tested for blocking of T agglutination. Nine agglutinated well in the presence of T antibody, and 8, like the type 6 matt virulent culture described above, were not agglutinable. Whether these strains lacked T substance, or whether the T antigen was merely blocked could only be determined by further studies of the antigenicity of these strains and of their ability to absorb T antibody.

C. Identification of M and T Components of Group A Hemolytic Streptococci.—It is possible by the several tests at present available to ascertain the antigenic composition of a given strain with respect to M and T antigens.

These tests have been applied to all available strains of several types. A comparison of the agglutination reactions of various type 14 strains tested is given in Table VIII as an example of the results obtained, together with the corresponding precipitin reactions with extracts of these strains. In most instances agglutination in anti-M serum corresponded with the presence of M substance. Some strains, however, probably due to differences in sensitivity of the suspensions, agglutinated to higher titers in anti-M serum than others which, according to the results of precipitin reactions, contained more M substance.

As a result of similar studies, 28 strains apparently lacking T antigen have been found in types 14, 17, 19, 24, 26, 28, 29, and 30. At least one strain in each type received sufficient investigation to indicate that T antigen was lacking (as in strain C 630, type 14) and not simply masked (as in strain S 43, type 6). Although the data so far collected are too few to be statistically significant, they suggest that strains containing both M and T antigens occur more frequently than those containing only one of these antigens.

DISCUSSION

During the study of the effect of proteolytic enzymes on group A hemolytic streptococci, it was observed that certain strains, classified in a given type by the M precipitin reaction, did not appear to contain the T antigen characteristic of that type. The experiments described in this paper show that these strains are not agglutinated by T antibodies, nor do they absorb anti-T agglutinins. Furthermore, immunization of rabbits with them does not induce T antibody formation. Since T antibodies usually appear early in the course of immunization, the fact that none appeared at any time in response to immunization with these unusual strains led to the conclusion that they did not contain T antigen.

Certain other strains, known from previous study to contain T antigen, were also found to be inagglutinable in anti-T serum. With these strains, however, this failure to agglutinate was correlated with the presence of large amounts of

M antigen in variants made virulent by serial passage through mice. Susceptibility to T agglutinins appeared when these cultures were subjected to slightly unfavorable environments. The possession of T antigen by these strains was also readily demonstrated by immunization of rabbits and by absorption experiments. A similar blocking of the agglutination reactions due to one antigen by the presence of another has been observed with other bacteria, for example, with the Vi antigen of the typhoid bacillus (3-5). Blocking of M agglutination reactions in type 1 has been previously reported (1). In such instances it has not been determined whether the T antigen or unknown factors prevent M agglutination.

An analysis of the antigenic composition of the available strains has been made in a limited number of types. Matt strains containing both M and T antigens appear to occur more frequently than matt strains containing M antigen alone or than glossy strains containing T antigen alone. The number of matt or glossy strains found in any series will probably depend upon the source of the cultures, since strains isolated from active infections usually contain M substance, and degraded glossy variants are not infrequently recovered from healthy carriers.

It is known that M substance is related to virulence and that the capacity of a given strain to produce this substance may vary, ranging from the large amount produced by matt strains to the trace usually demonstrable in their glossy derivatives. On the contrary, T substance is unrelated to virulence and the capacity of a strain to produce this antigen seems to be constant. At the present time the function of T antigen is not understood.

In many epidemiological studies the identification of streptococci has been based solely on T agglutination. The occurrence of strains devoid of T substance or those in which this antigen is blocked may in some instances lead to erroneous conclusions. Although it is possible to identify the type of such strains by means of M agglutination or M precipitin reactions, the determination of T content also may be of value in distinguishing apparently identical strains of the same type, as well as in partially identifying glossy strains. In careful epidemiological surveys, therefore, it may prove advisable to study both the M and T antigens of the group A hemolytic streptococci recovered.

SUMMARY

1. Group A hemolytic streptococci have been described which do not agglutinate in anti-T serum because they lack T antigen.
2. Other strains occur which contain T antigen yet do not agglutinate in anti-T serum because T agglutination is blocked by the presence of large amounts of M substance in the streptococci.
3. Strains belonging to a number of types have been analyzed to determine their antigenic composition with respect to M and T antigens.

BIBLIOGRAPHY

1. Lancefield, R. C., *J. Exp. Med.*, 1940, **71**, 521.
2. Lancefield, R. C., *J. Exp. Med.*, 1943, **78**, 465.
3. Felix, A., and Pitt, R. M., *Lancet*, 1934, **2**, 186.
4. Felix, A., and Pitt, R. M., *J. Hyg.*, Cambridge, Eng., 1935, **35**, 428.
5. Bhatnagar, S. S., Speechly, C. G. J., and Singh, M., *J. Hyg.*, Cambridge, Eng., 1938, **38**, 663.