OCCURRENCE OF R ANTIGEN SPECIFIC FOR GROUP A TYPE 3 STREPTOCOCCI*

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Three different protein antigens, designated by the letters M, T, and R, have been shown to be present on the surface of Group A streptococci (1). With respect to streptococcal infections the M antigens are the most important, since they are essential to the virulence of the microorganisms and stimulate the production of protective antibodies in the infected or immunized animal. Accordingly, the M antigens were selected as the basis for classification of Group A streptococci into serological types.

The serological type of a given Group A streptococcus is usually determined by specific precipitin tests for M antigen. Other immunological reactions dependent upon this antigen-antibody system can also be used for identification of either M antigen or its antibody. Thus, under appropriate conditions the following procedures have been used to obtain information about the type-specific reactions of M antigens and their antibodies: agglutination reactions, active and passive protection tests, anaphylaxis experiments, bactericidal tests dependent upon phagocytosis of sensitized streptococci, and occasionally complement fixation tests (2).

In those cases in which precipitin or agglutination tests give equivocal results in attempted identification of the M antigen, definitive information can be derived from the more biological mouse protection and bactericidal tests. Thus, T antigens, which cause confusion in agglutination tests for type specificity, can be clearly differentiated from M antigens by protection or bactericidal tests. Similarly, these procedures serve to establish the fact that 28 R antigen is not one of the M proteins.

The present report concerns a newly identified antigen found in certain Type 3 strains. The experiments reported in this paper show that it is not an M antigen. So far it has not been found in any other type or group of streptococci, but only in Group A streptococci, Type 3. Since this antigen, like the 28 R antigen, appears to represent another protein antigen which can interfere

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with the identification of M protein by the precipitin test, it has been designated 3 R antigen.

Methods

Streptococcal Strains.—Two hundred and four strains of Group A, Type 3 streptococci in our stock of frozen and dried cultures collected during the past 40 years were examined. Not all of these are included in Table I because of duplication by repeated cultures from the same patient or the recovery of a single strain from many individuals in large epidemics in military establishments.

Strains of special interest were Griffith's Type 3 representative strain, Lewis opaque (designated T3 in this laboratory), Colebrook's strain Richards (D58 in these records), and a variant strain, D58X, which contains no M antigen; strain S80 from one of the Texas army camps of 1918; Dochez's strain C203, widely used in many laboratories. In strain designations throughout this paper the figure preceding the slanted line is the strain number, and the figure following the slanted line indicates the number of mouse passages, unless otherwise indicated.

Antisera.—Antisera containing only type 3 M antibody were obtained: (*a*) by immunizing rabbits with strains which did not contain Type 3 R antigen, for example, strain B930/24, and absorbing the sera in the usual way to remove all except anti-M antibodies; (*b*) by using antisera from rabbits immunized with a strain which contained both Type 3 M and Type 3 R antigens, for example, strain D58; and in addition to the usual absorption for preparing type-specific antisera, absorbing specifically with a strain which contained 3 R antigen but no 3 M antigen (for instance, strain D58X).

Antisera containing only 3 R antibodies were prepared by the same general methods:

(*a*) Rabbits were immunized with strain D58X, which contained Type 3 R but no type 3 M antigen, and absorbed with heterologous type strains so that they contained only precipitins for Type 3 R antigen.

(*b*) Sera from rabbits immunized with the parent strain D58 were absorbed with strain B930/24 to remove precipitins for Type 3 M antigen, leaving precipitins for 3 R antigen.

Immunological Reactions.—The technique used for the bactericidal test has been described in detail recently (2). Other immunological tests are described in the same and in a preceding paper (3).

In the present studies cultures for virulence and protection tests were the rapidly growing 2 hour cultures also used for the bactericidal tests.

EXPERIMENTAL

The presence of 3 R antigen, was not discerned until a variant containing this antigen but lacking Type 3 M antigen was obtained in a culture made from a single colony selected for an unrelated purpose. Antiserum prepared by immunizing rabbits with this single colony strain and then absorbed in the usual way showed very strong precipitin reactions with extracts of the homologous strain, D58X, and with extracts of certain other Type 3 strains tested. It developed, however, that strain D58X no longer reacted in standard Type 3 anti-M serum,[1] but appeared to represent some undescribed type.

[1] Dr. Elaine L. Updyke very kindly brought this fact to my attention. Dr. Hideo Kusama of the National Institute of Health in Tokyo has informed me that he also has encountered these two kinds of type 3 strains.

Occurrence of Type 3 R Antigen.—Examination of the precipitin reactions of a large number of Type 3 strains showed that many of these streptococci contain both the standard Type 3 M antigen and the 3 R antigen characteristic of strain D58X. A number of strains contain only Type 3 M antigen, or only 3 R antigen, and some contain predominantly 3 R antigen and only a trace of Type 3 M antigen. These findings are summarized in Table I.

The number of strains in each category can only be taken to represent approximate distribution, since several large wartime epidemics are included. The second column contains strains from a single epidemic in a training camp. The properties of several representative strains were studied in detail.

Biological Properties of the 3 R Antigen.—Bactericidal and mouse protection tests were employed to find out whether the antigen in strain D58X was concerned with virulence and protection, as is the case with M antigens,

TABLE I

Occurrence of Type 3 M and R Antigens in Group A, Type 3 Streptococci

*Antigenic components of Type 3 stock strains	No. of strains	
	Various sources	Single epidemic in one military camp
Type 3 M antigen only	26	22
Type 3 M and 3 R antigens	26	56
Predominantly 3 R antigen	5	
Type 3 R antigen only	13	8

* Determined by precipitin reactions.

or whether it was inert in these respects and, therefore, similar to the R antigen (28 R antigen) previously studied.

Bactericidal tests with strain D58X showed that it would not grow when rotated in normal human blood under the conditions of this test even with a very large inoculum, and only a small percentage of organisms survived to the end of the experiment. Furthermore, only a slight inhibitory effect was found when either Type 3 anti-M serum or antiserum against strain D58X itself was added to the system. In the stationary control, normal growth occurred because the leukocytes were not in constant contact with the streptococci. The results are recorded in Table II.

It is obvious that strain D58X behaved like a glossy strain without M antigen, readily phagocyted in this system, and that the D58X antigen and its antiserum were without effect in this test. Four other strains without M antigen and four with only a trace of M antigen, all however containing the 3 R antigen, failed to grow in normal human blood and behaved like typical glossy strains.

On the other hand, all strains selected for bactericidal tests on the basis of their high content of Type 3 M antigen grew well from small inocula in normal human blood; and the growth of all was inhibited in the presence of Type 3 anti-M antibodies but was unaffected by serum containing antibody for the 3 R antigen from rabbits immunized with strain D58X. Six of

the strains employed had Type 3 M antigen only, and 4 had both antigens. There was no difference in their behavior in these bactericidal tests. (See Table III for typical examples.)

Since strain D58X appeared to be a glossy derivative of a strain which originally had both M and R antigens, it was passed through a series of mice to see whether it would regain its M antigen. After 11 mouse passages, extracts showed strong M precipitin reactions with

TABLE II

Poor Growth of Strain D58X in Normal Human Blood in Bactericidal Tests and Lack of Specific Inhibition with Immune Sera

Strain D58X tested (contains 3 R antigen but no Type 3 M antigen)	Results of bactericidal tests No. of colonies			
No. of streptococci inoculated	*Inoculum*			
	∞	4000	194	15
No. of streptococci at end of test with	*Bactericidal tests*			
(*a*) Normal rabbit serum	500	300	5	0
(*b*) D58X Pool 9 antiserum (anti 3 R)	300	14	0	0
(*c*) Type 3 anti-M serum	200	7	2	0
(*d*) Stationary control: Normal rabbit serum	∞	∞	2000	500

Technique of Indirect Bactericidal Test.—

Suitable serial dilutions of culture were made for the inoculum. 0.1 cc. of each dilution was plated in a blood agar pour plate to determine the number of streptococci inoculated.

Each tube of the bactericidal test contained:—

0.05 cc. serum or plasma.

0.1 cc. culture dilution (several dilutions were used for each test).

0.3 cc. heparinized normal human blood.

After 3 hours rotation at 37°C., 0.1 cc. sample from each tube was plated in blood agar pour plates and incubated over night.

In the stationary control, a duplicate of (*a*), phagocytosis did not occur because the leukocytes were not in constant contact with the streptococci.

The number of colonies which grew out in the pour plates from inoculum and test were counted or estimated to determine the number of streptococci in each tube at the beginning and end of the test.

∞ indicates innumerable colonies with blood completely hemolyzed.

4000 indicates innumerable colonies with some areas of unhemolyzed blood. Records of 2000 to 500 colonies were estimated by comparison. Colonies on plates with < 400 were usually counted.

absorbed type-specific Type 3 antisera, as well as strong precipitin reactions with absorbed D58X anti-3 R serum (3 R reactions). Extracts prepared from each of the 11 serial mouse passage cultures showed that the change had occurred during the 8th or 9th mouse passage. Passage cultures 1 through 8 were negative for Type 3 M antigen, and passages 9, 10, and 11 were strongly positive. All passage cultures were equally strong in their 3 R precipitin reactions in absorbed D58X antiserum. The glossy strain, D58X, had, therefore, recovered its M antigen as a result of mouse passage without any apparent change in its 3 R antigen.

Comparative virulence, mouse protection, and bactericidal tests, as well as precipitin tests, were made with strain D58X before and after mouse passage and with strain D121, a

Type 3 strain with little or no 3 R antigen. Virulence tests and precipitin reactions are shown in Table IV. The virulence of strain D58X was increased at least 100,000-fold by mouse passage and after 11 mouse passages, good M precipitin reactions were obtained which were as strong as those with a known Type 3 strain, D121. The 3 R precipitin reaction of the mouse passage strain, D58X/11, in serum containing only 3 R antibody (D58X Pool 9) was

TABLE III

Growth of Streptococci Containing Type 3 M Antigen in Normal Human Blood in Bactericidal Tests

Inhibition with Type 3 anti M serum but none with anti-3 R serum

Representative Type 3 strains tested		Results of bactericidal tests: No. of colonies			
Strain B930/24 (Type 3 M antigen, no R antigen)	No. of streptococci inoculated	*Inoculum*			
		136	55	12	1
	No. of streptococci at end of test with	*Bactericidal tests*			
	(*a*) normal rabbit serum	∞	∞	4000	750
	(*b*) Type 3 anti-M serum	1	0	0	0
	(*c*) D58X Pool 9 (anti-3 R) serum	∞	4000	4000	500
*Strain C203/42 (Type 3 M antigen, no R antigen)	No. of streptococci inoculated	*Inoculum*			
		171	34	8	2
	No. of streptococci at end of test with	*Bactericidal tests*			
	(*a*) normal rabbit serum	∞	4000	500	1
	(*b*) Type 3 anti-M serum	3	0	0	0
	(*c*) D58X Pool 9 (anti-3 R) serum	∞	4000	750	400
Strain C199 (Type 3 M antigen and 3 R antigen)	No. of streptococci inoculated	*Inoculum*			
		239	72	18	1
	No. of streptococci at end of test with	*Bactericidal tests*			
	(*a*) normal rabbit serum	∞	∞	4000	750
	(*b*) Type 3 anti-M serum	25	4	0	0
	(*c*) D58X Pool 9 (anti-3 R) serum	∞	4000	215	0

See Table II for details of experiment.

* A Type 3 strain which contains Type 1 T antigen.

unchanged after mouse passage. Strain D121 was essentially negative in precipitin reactions with this serum, and probably has no 3 R antigen.

Passive protection tests with these strains and antisera are recorded in Table V. Even enormous numbers of organisms of strain D58X contained in 10^{-4} and 10^{-5} cc. failed to kill mice, and the Type 3 anti-M and anti-3 R sera afforded no protection to the mice which were killed in doses of 10^{-1}, 10^{-2}, and 10^{-3} cc. After mouse passage and recovery of Type 3 M antigen, however, strain D58X/11 killed mice in small doses, and only Type 3 anti-M serum protected mice against infection. The results were almost identical with those obtained at the same time with strain D121.

Bactericidal tests with these same cultures were performed on the same day as the protection and precipitin tests. The results with strain D58X are recorded in Table II. Those

with strain D58X/11 and D121 are not tabulated, but they were essentially the same as the bactericidal reactions of the three strains recorded in Table III. In serum titrations, the Type 3 anti-M serum employed was still highly inhibitory in a dilution of 1:100, but showed decreasing activity in a 1:200 dilution. The D58X serum (anti-3 R) usually showed no inhibitory effect even with undiluted serum. The occasional positive effect with this serum was probably due to traces of Type 3 M antibody in the serum, since glossy strains (such as D58X) often elicit a slight type-specific response when injected into rabbits repeatedly for considerable periods of time.

TABLE IV

Effect of Mouse Passage on Strain D58X

Strains tested	Mouse virulence					Precipitin tests	
	Dose of culture, *cc.*					HCl extracts and	
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Type 3 anti-M serum	Anti-3 R serum
Strain D58X							
(*a*) before mouse passage	*S	S	S	S	S	−	++++
(*b*) after 11th mouse passage	15	15	15	24	20	++++	++++
Strain D121	15	15	15	21	40	++++	− or ±
Colony counts of inocula	No. of colonies in pour plates of each dose						
Strain D58X		∞	444	48	2		
" D58X/11		∞	4000	120	16		
" D121		∞	364	47	8		

*S indicates survival of mouse for 10 days.

Numerals indicate approximate number of hours to death of mouse. All mice receiving 10^{-1}, 10^{-2}, or 10^{-3} cc. of culture died within 15 hours.

Cultures taken at autopsy from mice receiving 10^{-1}, 10^{-2}, or 10^{-3} cc. of strain D58X did not contain Type 3 M antigen. These mice died apparently as a result of the overwhelming dose of glossy organisms.

See reference 3 for details of test.

Precipitin tests recorded on a − to ++++ scale.

The M precipitin reactions were correlated with virulence and protection results, as well as with the results of the bactericidal tests. If Type 3 M antigen was present, as in strains D58X/11 and D121, the culture behaved like a typical matt culture in all these tests. Without Type 3 M antigen, as in strain D58X, the culture was avirulent and failed to grow in normal human blood in the bactericidal test. The presence or absence of 3 R antigen did not affect any of these reactions (Tables III to VI).

Properties of 3 R Antigen.—The 3 R antigen and its antibody differ, therefore, from M antigen and its antibody in being unrelated respectively to virulence and protection. The 3 R antigen is also different from M antigen in the effect upon it of heat, especially at pH 2. Solutions heated at this pH

in a boiling water bath for 10 minutes, and then neutralized, no longer precipitated with antiserum containing 3 R antibody. Heating at pH 8 was also deleterious but less so than at pH 2. These effects of heating were similar

TABLE V

Passive Mouse Protection and M-Precipitin Tests with Strain D58X before and after Mouse Passage

Strains and sera used in protection and precipitin tests		Passive mouse protection tests					Precipitin tests with HCl extracts diluted 1:				
		Dose of culture, cc.									
Strain	Serum	*10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	1	2	4	8	16
D58X	(*a*) Type 3 anti-M	15	S	S			−	−	−	−	−
"	(*b*) D58X pool 9 anti-3 R	S	S	S			++++	+++	+++	++	+
"	(*c*) normal rabbit	S	S	S							
"	(*d*) none	15	S	S							
D58X/11	(*a*) Type 3 anti-M	15	S	168	S	S	++++	+++	++	+	+
"	(*b*) D58X pool 9 anti-3 R	21	15	39	25	39	++++	+++	++	++	+
"	(*c*) normal rabbit	15	15	22	24	28					
"	(*d*) none	15	15	15	27	S					
D121	(*a*) Type 3 anti-M	25	72	168	S	S	++++	+++	++	±	±
"	(*b*) D58X pool 9 anti-3 R	15	15	15	15	48	±	−	−	−	−
"	(*c*) normal rabbit	15	15	15	15	48					
"	(*d*) none	15	15	15	48	48					
Colony counts of inocula		No. of colonies in pour plates of each dose									
Strain D58X		∞	∞	760							
Strain D58X/11/1				142	15	3					
Strain D121				444	62	5					

See Table IV for details of tests.

* All mice receiving 10^{-1} and 10^{-2} cc. of culture died within 15 hours.

Bactericidal tests with these same cultures were performed on the same day. Results with strain D58X are those recorded in Table II. Results with strains D58X/11 and D121 are essentially the same as those of the three strains recorded in Table III.

to the effects of heating 28 R antigen and were in contrast to the much slighter destructive effect of heat on solutions of type 3 M antigen or of M antigens of other types.

Unlike 28 R antigen, Type 3 R antigen was not obtained in extracts made by heating the streptococci in phosphate buffer at pH 8 for 10 minutes in

a boiling water bath. Digestion with trypsin destroyed the serological activity of 3 R antigen, whereas 28 R antigen was liberated from the streptococcal cell by this enzyme. The 3 R antigen is similar to the 28 R antigen in that its serological activity was destroyed by peptic digestion.

Effect of Mouse Passage on Antigens of Degraded Glossy Strains Containing 3 R Antigen.—Strain D58X, which contains no M antigen, and 4 other strains

TABLE VI

Effect of Loss of Type 3 M Antigen on Virulence and Ability of Streptococci to Grow in Normal Human Blood in Bactericidal Tests

Strains	Mouse virulence						Bactericidal tests				Precipitin tests	
	*Dose of culture, *cc.*						Ability to grow in normal human blood. Varying culture dilutions No. of colonies				HCl extracts and	
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}					Type 3 anti-M serum	Anti-3 R serum
C199 matt	15	39	51	S	24	47	§∞	4000	700	500	++++	+++
C199 glossy	S	S	S	S	S	S	§ 0	0	0	0	−	+++
D121 glossy	‡39	S	S	S	S	S	§96	1	0	0	−	±
Colony counts of inocula	No. of colonies in pour plates of each dose used for mice						No. of colonies in pour plates of each dilution of inoculum					
Strain												
C199 matt				71	10	1	49	12	4	3		
C199 glossy				103	16	0	86	21	3	2		
D121 glossy				144	16	2	∞	2000	62	11		

See previous tables for details of tests.

* Mice receiving 10^{-1} or 10^{-2} cc. of culture all died within 15 hours.

‡ The culture obtained from this mouse at autopsy showed Type 3 M antigen in the precipitin test. See Tables IV and V for virulence tests with original matt variant of strain D121.

§ Duplicate control series, kept stationary at 37°C. during rotation of the tubes in the bactericidal tests, all grew as well as the C199 matt series.

Tests, not tabulated here, in which Type 3 anti M serum was added to the C199 matt series, together with appropriate controls, resulted in almost complete inhibition of growth of the sensitized streptococci, just as with the parent strain, C199 (See Table III).

which had 3 R antigen but no M antigen, were passed in series twice daily through mice to investigate whether strains containing M antigen would be obtained and, if so, whether the M antigen would be Type 3 or possibly some other serological type. After 8 to 16 mouse passages all 5 strains yielded cultures with large amounts of Type 3 M antigen. There was no evidence of change in the 3 R antigen of these cultures after mouse passage. The identification of Type 3 M antigen in strain D58X/11 was made by means of precipitin reactions, mouse virulence and protection tests, and bactericidal tests, as

described above. For the other strains the Type 3 M antigen was identified only by precipitin reactions which were in each case strong and unequivocal. The evidence indicates, therefore, that all glossy strains containing the 3 R antigen were converted to Type 3 M-containing strains as a result of mouse passage.

Loss of Type 3 M Antigen by Subculturing in Plain Broth.—A procedure which was the reverse of converting a glossy to a matt variant as just described was the degradation of virulent matt strains containing Type 3 M antigen to avirulent glossy variants without this antigen. This was accomplished by daily serial subcultures in plain broth.

Strain D121 which was used for comparison with strains D58X and D58X/11, Table IV, lost its M antigen after 10 subcultures. An extract gave no reaction with Type 3 anti-M serum. The trace of reaction with 3 R antiserum (absorbed D58X antiserum) which was present in the original culture remained unchanged. The virulence for mice of the D121 variant was greatly reduced, and the strain was unable to grow in normal human blood under the conditions of the bactericidal test (see Table VI). A virulence test with the original strain, D121, is recorded in Table IV. Four other Type 3 strains, three with 3 R antigen and one lacking this antigen, were similarly degraded by 25 subcultures in plain broth. Strain D58X/11 and the original D58 strain were among those employed.

One of the cultures used, strain C199, when streaked on the surface of a blood agar plate after the first subculture in plain broth showed two distinct kinds of colonies. They were typically matt and glossy in appearance. Subcultures of these two varieties of colonies were tested with the following results: The matt colony had all the characteristics of an M-containing Type 3 organism. The glossy colony had no M substance, was avirulent for mice, and would not grow in normal human blood in bactericidal tests. The results are recorded in Table VI. These 2 variants maintained their characteristics in stock cultures, but with continued subculturing in plain broth the parent strain has after 7 subcultures also become degraded and lost its M antigen. The 3 R antigen in all of these strains has apparently remained unchanged.

Table VI illustrates the correlation between the M antigen of the matt variant of strain C199, its high degree of mouse virulence, and the failure of normal human leukocytes to phagocytize these M-containing streptococci in bactericidal tests. In contrast is the low degree of virulence of the two degraded glossy variants derived from strains C199 and D121, their lack of resistance to phagocytosis in normal human blood, and the absence of M antigen in extracts prepared from them. The failure of 3 R antigen to affect the virulence of these organisms or their ability to grow in normal human blood without antibody was also demonstrated.

A study of the mechanism of matt-to-glossy variation of Type 3 strains in the throats of normal children in a residential school was reported by Wormald (4) following the observations particularly of Todd (5) and of Rothbard and Watson (6). The usual replacement of the matt M-containing form by glossy variants without M was thought to be due to selective survival of randomly occurring mutants depending on differences in growth rates and

differential nutrient value of the micro environment. He compared these *in vivo* changes with the gradual replacement of matt colonies *in vitro* during daily serial transfers in glucose broth. These changes occurred in all type 3 strains tested. Comparable studies with Type 12 strains gave the same results. Observations in this laboratory have indicated that in some strains or types, M antigen is rapidly lost under laboratory conditions, although in others it persists even under adverse conditions of growth.

DISCUSSION

The M precipitin test with absorbed type-specific antisera and crude HCl-extracts of Group A streptococci has been in use for many years. This method has proved satisfactory for demonstrating the presence of the type-specific M antigen and determining the serological type of a given strain of streptococcus.

In the early work of establishing the occurrence of specific serological types among streptococci pathogenic for man, reliance was placed on the standard passive protection test in mice (7). When it became evident that this biological test was dependent upon the M antigen and its antibody, it was demonstrated that the results of the M precipitin reaction paralleled those of the mouse protection test (8). The simpler *in vitro* precipitin and agglutination tests, therefore, were adopted for ordinary type classification of Group A streptococci, and the *in vivo* mouse protection test was only used for special studies in which precipitin and agglutination reactions gave ambiguous results. Recently the bactericidal test for phagocytosis of streptococci in the presence of homologous type-specific immune serum, which contains anti-M antibodies, has been found useful as another biological test for the M anti-M system (2, 10).

In the past the presence of two or more protein antigens other than the M antigen have caused confusion in typing Group A streptococci *in vitro*. Among these are certain trypsin-sensitive substances described by Maxted in acid extracts of Group A, Type 2 streptococci which are not M substances (11). Hambly has reported somewhat similar findings for Type 4 streptococci (12). According to the results obtained by these investigators, the antigens which they have studied may be T antigens, although some of the properties of these proteins are different from those characteristic of T antigens.

The T antigens, so called on account of their frequent relationship to type-specific agglutination as determined by the test tube technique or by Griffith's classical slide agglutination method, are not usually specific for a single type as determined by M precipitin and mouse protection tests. Thus a single T antigen, or possibly serologically related substances, may be found associated in streptococci with M antigens of several different types, for example, Types 4, 24, 26, 29, 46, and 48. Occasionally, a single M antigen may be as-

sociated with more than one T antigen, as in Type 12. Rarely, a T antigen occurring in a Group A type has also been found in a type within another streptococcal group, for example, as noted by Griffith, Group A, Type 2 and Group C, Type 21 (9).

R antigen, however, which was first found in Group A Type 28 strains, occurs in some members of three types of Group A streptococci, as well as in certain strains of Groups B, C, and G (13). Originally this antigen, on the basis of precipitin reactions which seemed to indicate that it was an M antigen, was considered to be the type-specific antigen of Type 28. Eventually, by use of mouse protection and bactericidal tests, the 28 R antigen was shown to be unrelated to virulence and protection, and the real M antigen of Type 28 which had these fundamental properties was identified (2).

The properties of the 3 R antigen, described in this paper, are not identical with those of the 28 R antigen. Thus, the two R antigens differ in their susceptibility to tryptic digestion and in their extractability with heat at pH 8. However, to some extent both have in common with M protein the property of extractability with heat at pH 2, and consequently both can give rise to confusion in typing by the precipitin technique. It seems preferable, therefore, to assign the letter R to the new Type 3 antigen rather than using a new letter for its designation.

Type 3 R antigen has not been found except in Type 3 streptococci, and it is unrelated to the phenomena of virulence and protection by which M antigens are defined. On the other hand, Type 3 **M** antigen is necessary for the virulence of Type 3 strains, for their ability to grow in normal human blood by resisting phagocytosis in bactericidal tests, and for stimulating protective type-specific antibody following immunization or infection.

Approximately one-half of the Type 3 strains examined have 3 R antigen. A number of old stock strains which had this antigen without having demonstrable M antigen reverted during serial mouse passage to M-containing strains, in every case Type 3. Loss of M antigen, occurring spontaneously or induced by subculture in unfavorable media had no detectable effect on the cellular content of 3 R antigen. The finding of a second serologically distinct R antigen in Group A streptococci suggests the possibility of a more widespread occurrence of such streptococcal antigens than had been previously suspected.

SUMMARY

Approximately 50 per cent of Group A, Type 3 streptococci contain a hitherto undescribed antigen found only in Group A, Type 3 organisms. It is serologically distinct from Type 3 M antigen and is designated as 3 R antigen.

Strains containing 3 R antigen but no Type 3 M antigen are "glossy," avirulent Type 3 variants. These strains can be obtained by repeated trans-

fers of virulent M-containing streptococci in artificial media under unfavorable conditions of growth. These degraded streptococci recover Type 3 M antigen during serial passage through mice. The amount of 3 R antigen in a strain is not affected by a decrease or increase in M antigen.

The 3 R antigen is unrelated to virulence. Antibodies to this antigen do not protect mice against infection or promote phagocytosis in bactericidal tests. The 3 R antigen-antibody system can give rise to confusion in M-precipitin reactions. In all these properties 3 R antigen is similar to 28 R antigen, although in certain other properties the two R antigens are not identical. They are serologically distinct.

Virulence and ability to grow in normal human blood under the conditions of the bactericidal test are correlated with the presence of M antigen of Group A, Type 3 streptococci. Mouse protection and specific inhibition of growth of Type 3 streptococci by phagocytosis in bactericidal tests are associated with the presence of Type 3 anti-M antibodies.

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