Further Studies of Some "Nontypable" Group A Streptococci

ROBERT W. QUINN,2,3 W. R. MAXTED,4 AND P. N. LOWRY2

Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, and Cross-Infection Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England

Received October 2, 1975

Thirty-two strains of group A hemolytic streptococci which could not be M typed with the available typing sera in Nashville, Tenn., were reinvestigated at the Streptococcus Reference Laboratory in Colindale, England, in order to estimate the efficacy of other antisera not available in Nashville and newer techniques (the opacity factor inhibition test) of typing strains not isolated in England. Fifty percent were eventually typed and all but four contained enough M protein to suggest that they would have been typed had the appropriate typing sera been available. The results indicate that group A streptococci truly lacking M protein were seldom isolated from the Nashville children from whom the streptococci were cultured. Several factors responsible for nontypability were considered, including the nonavailability of the necessary type-specific antisera and loss of M protein due to a change from Matt to glossy colonial types in the laboratory.

INTRODUCTION

A recent report concerning some characteristics of a collection of nontypable group A streptococci recovered from throat cultures of patients in Nashville, Tenn. (1), concluded that these strains caused streptococcal disease in spite of the finding that the majority of them did not possess identifiable M protein, a determinant of virulence in human infections. Of 53 of these so-called nontypable group A streptococci, 17 became typable with available M typing sera during the course of mouse passage. Of the remaining 36, 18 not belonging to any of the available types were able to stimulate M antibody production in rabbits. However, 12 failed to promote type-specific antibody and remained negative for evidence of M protein.

METHODS

Following publication of these results (1), one of the investigators (WRM) investigated some of the strains which originally could not be typed with the available typing sera. This study was initiated in order to estimate the usefulness of other antisera and newer techniques in typing strains of group A streptococci not isolated locally. Also, this was an attempt to see if it were possible to estimate the amount of M-associated protein (MAP), an acid-extractable streptococcal antigen shown to neutralize several M antisera (2), in order to learn whether the strains contained sufficient antigen to offer some prospect of typing them without the need of animal passage. Accordingly, 32 strains which could not be typed with the available M-typing sera in

1This research supported by USPHS Grant 2 RO1A107438 and by General Research Support Grants.
2Vanderbilt University School of Medicine.
3Address reprint requests to Dr. Quinn.
4Cross-Infection Reference Laboratory.
Nashville were sent lyophilized to the Streptococcus Reference Laboratory, Colindale, England.

An attempt was made to make an initial broad division of the nontypable strains into opacity factor (OF) +ve and OF –ve and to type the OF +ve cultures by means of the OF-inhibition test (3). In this test OF, a type-specific product of certain group A streptococci which causes opacification of horse serum, is neutralized by specific antisera.

M and T typing were carried out on all strains using antisera prepared at the Colindale laboratory. Finally, the extracts of the strains were tested for the content of MAP. A small number of strains was also tested before and after passage through normal human blood to see if the M content was increased; others were passed through mice.

**RESULTS**

Of the 32 strains studied, 14 strains were OF –ve, and among these 3 were M typable and 11 were untypable. Of the 32 strains, 18 were OF +ve; 4 strains among the OF +ve were M typable; 12 were typed by means of the OF-inhibition test; and 6

| Strain number | Opacity factor | T reaction | M type (any method) | Highest MAP titer observed |
|---------------|----------------|------------|---------------------|---------------------------|
| 1             | +              | 12         | 22                  | 80                        |
| 2             | –              | Imp 19     | 53                  | 20–40                     |
| 3             | –              | Imp 19     | 53                  | 160                       |
| 4             | –              | Imp 19     | 53                  | 80                        |
| 5             | +              | Imp 19     | 59                  | 40–80                     |
| 6             | +              | Imp 19     | 59                  | 80–160                    |
| 7             | +              | 12         | 62                  | >320                      |
| 8             | +              | 25/Imp 19  | Provisional 3354    | 40–80                     |
| 9             | +              | 25/Imp 19  | Provisional 3354    | 40–80                     |
| 10            | +              | 11         | Provisional Constable | 40–80                   |
| 11            | +              | 11         | Provisional Constable | 40–80                   |
| 12            | +              | 11         | Provisional Constable | 80–160                  |
| 13            | +              | Imp 19     | Provisional Higgins | 80                       |
| 14            | +              | Imp 19     | Provisional Higgins | 80                       |
| 15            | +              | 25/Imp 19  | Provisional Higgins | 320                      |
| 16            | +              | –          | –                   | 80                       |
| 17            | +              | –          | –                   | <10                      |
| 18            | +              | –          | –                   | 160                      |
| 19            | –              | 14         | –                   | 40–80                    |
| 20            | –              | Imp 19     | –                   | 40–80                    |
| 21            | –              | –          | –                   | 80–160                   |
| 22            | –              | 28         | –                   | <10                      |
| 23            | –              | –          | –                   | 80–160                   |
| 24            | +              | 3          | –                   | 320                      |
| 25            | –              | Imp 19     | –                   | 160–320                  |
| 26            | +              | –          | –                   | 80–160                   |
| 27            | –              | –          | –                   | <10                      |
| 28            | –              | 3          | –                   | 40–80                    |
| 29            | +              | Imp 19     | –                   | 80–160                   |
| 30            | –              | 9          | –                   | <10                      |
| 31            | –              | Imp 19     | –                   | 80                       |
| 32            | –              | –          | –                   | 80                       |
were nontypable. Among the established M types found were 3 type 53, 1 type 22, and 1 type 62; 8 belonged to three provisional types in the Colindale Laboratory, 2 type “3354,” 3 type “Constable,” and 3 type “Higgins” (Table 1).

Of the remaining 17 nontypable strains, the 11 OF –ve strains appeared to be different from the 6 OF +ve strains; among these two latter categories, the T-agglutination reactions separated most of them from each other and from those that gave no agglutination reactions at all.

It is reasonable to suppose that streptococci capable of surviving passage through normal human blood have adequate amounts of M antigen. Of 8 nontypable strains which were passed through normal human blood at Colindale, the M content as estimated by the MAP titer of an acid extract increased in 4 but remained the same in 2. Two strains failed to grow in human blood, never gave significant MAP titers, and were considered to be devoid of M protein. Strain 18 had an MAP titer of 20 and after passage through normal human blood, 160, but it remained nontypable with typing sera available at the reference laboratory.

Six pairs of strains that were nontypable before and after mouse passage when tested in Nashville (1) were reinvestigated in Colindale. Four reacted well with type 22 M antiserum, and their opacity factor was inhibited by type 22 OF antiserum. One passaged strain was not viable on arrival. Of the remaining two pairs, neither was M typable nor OF inhibited by type 22 antiserum (Table 2). Titrations of the M-associated protein of each pair were similar, and the prepassaged strain had enough M protein for the purpose of M typing. Since type 22 antiserum was not available in Nashville, the explanation of the nontypability of these six strains is obvious. The remaining two nontypable strains gave a strong agglutination reaction with type 8 T antiserum and were OF +ve, suggesting that they may well have been of that type; however, since type 8 M antiserum was not available in either Nashville or Colindale, this supposition could not be proved.

A summary of the findings with all 38 strains investigated (the original 32 and 6 used for mouse passage) is shown in Table 3. Half of the strains were typable. Of the 16 OF + strains that were typed, only 5 could be typed by the M-precipitation technique while all 16 were typed by the OF-inhibition test.

Acid extracts of all the strains that were typable were shown, as expected, to have

| Strain number | Opacity factor | M type | OF inhibited by serum of type | MAP titer of extract |
|---------------|--------------|--------|-----------------------------|---------------------|
| 33            | +            | 22     | 22                          | 160                 |
| 33 Passaged   | +            | 22     | 22                          | 80                  |
| 34            | +            | 22     | 22                          | 160                 |
| 34 Passaged   | +            | 22     | 22                          | 160                 |
| 35            | +            | 22     | 22                          | 160                 |
| 35 Passaged   | -            | 22     | 22                          | 160                 |
| 36            | +            | 22     | 22                          | 160                 |
| 36 Passaged   | +            | 22     | 22                          | 160                 |
| 37            | +            | 22     | 22                          | 160                 |
| 37 Passaged   | No hemolytic streptococci recovered | | | |
| 38            | -            | -      | Nil                         | 160                 |
| 38 Passaged   | -            | -      | Nil                         | 160                 |
TABLE 3
Characteristics of 38 Group A Streptococci Previously Found to Be Nontypable

| Number of strains | Number typed | Types identified | Inadequate MAP titer (<10) | Adequate MAP titer >20 |
|-------------------|--------------|------------------|---------------------------|------------------------|
|                   | Any method   | M                | OF-inhibition             |                        |
| 15                | 3            | 3                | /                         | M53 (3)                |
| OF +ve            | 16           | 5                | 16                        | 12                      |
|                   |              |                  |                           | 3                      |
| 23                |              |                  |                           | 12                     |
| Provisional types |              |                  |                           | 22                     |
| 3554 (2)          |              |                  |                           |                        |
| Constable (3)     |              |                  |                           |                        |
| Higgins (3)       |              |                  |                           |                        |
| Total             | 38           | 19               | 8                         | 16                     |
|                   |              |                  |                           | 19                     |
|                   |              |                  |                           | 4                      |
|                   |              |                  |                           | 34                     |

adequate titers of M-associated protein. However, 15 of the 19 nontypable strains (Table 1) also had a high MAP content, suggesting that these strains would be typable if the appropriate type-specific antisera were available. Only 4 of the nontypable strains had really low MAP titers (< 10) and were thus reckoned to be devoid of M antigen.

DISCUSSION

This work indicates that group A streptococci truly lacking M protein were seldom found on primary isolation in the Nashville children studied. Although all the strains were selected for study because they were nontypable, 50% were eventually typed and all but four had enough M protein present to suggest that they would have been typable had the appropriate antisera been available. The four strains that had an MAP titer of < 10 may well have been the result of cultural selection during their laboratory handling before and during the investigation.

The failure to type many group A streptococci is probably due to one of several factors, including the lack of the necessary type-specific antisera and low MAP titers resulting from a loss of M protein due to the change from matt (M +ve) to glossy (M -ve) colonies which occurs on subculture in the laboratory rather readily within some types. Some of the successfully typed strains belonged to well established M types (22, 53, 59, and 62) for which no antisera was available in Nashville. The others belonged to provisional new M types and were identified by means of the OF-inhibition test. This technique has proved consistently reliable as a supplementary typing system in England. In all cases where the OF-inhibition result could be checked by M-precipitation typing, there has been complete agreement. This was true with the five M type 22 strains included in this study.

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