Clinical Application of Immunofluorescence

III. Identification of Lancefield Group B Streptococci

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The identification of Lancefield group B streptococci with immunofluorescence was studied after significant differences between the precipitin and immunofluorescent results were noted. It was found that strains of group B streptococci, unlike groups A, C, and G streptococci, do not reliably react with a fluorescein isothiocyanate-labeled conjugate that contains antibodies mainly against C polysaccharide. It was assumed that the difficulty probably resulted from a blocking of the antigen-antibody reaction between the group antigen and its antibody by the S antigen located in the capsule. On the basis of this assumption, a conjugate was prepared that contained antibodies against the various S antigens of group B and it was found to be effective.

During a survey to determine the distribution of the serotypes of group A streptococci in West Germany (11), it was found that some non-group A strains that gave strong precipitation reactions with anti-group B serum gave weak immunofluorescence reactions with a sample of the same serum labeled with fluorescein isothiocyanate (FITC). This was just the opposite of the results noted by Kubin, Jelinkova, and Franek (5) in a comparison of precipitation and immunofluorescence methods for typing strains of group B streptococci. They were able to type correctly 90.9% of 152 group B strains with immunofluorescence. With the precipitin reaction, however, they were able to identify only 68.8% of the strains satisfactorily. The need for a clarification of this finding assumed even greater significance when it was noted that Smith (10) did not report a similar discrepancy between the precipitin and immunofluorescence reactions of groups A, C, and G streptococci and that Braunstein and coworkers (1) mentioned no difficulty with the application of immunofluorescence in the identification of group B streptococci. Consequently, when the opportunity arose to receive clinical cultures daily from the Munich-Schwabing Municipal Hospital, it was decided to study the problem. This paper is a report of the study that included the examination of 620 streptococcal cultures that were received during a period of 1 year.

MATERIALS AND METHODS

Streptococcal groups. Strains 090R (group B, type Ia), C-74 (group C), and D 166B (group G) were obtained from Rebecca C. Lancefield, Rockefeller University. The remainder of the major types of group B, H36B (type Ia), 18RS (type II), and M216 (type III), were obtained from W. R. Maxted, British Central Public Health Laboratory, London. In addition, a group B, type II strain that was isolated from an abscess was used as a "wild strain" antigen. The group A strain (B196) was isolated by Smith, Heymer, and Haferkamp (12) from the throat of a Bonn schoolchild who had no symptoms of a streptococcal angina. The antigens for immunizing rabbits were prepared by the method of Lancefield (6), with the exception that no attempt was made to destroy the group B type antigens.

Production of antisera. The immunization procedure utilized was also that of Lancefield (6). For antisera to groups A, C, and G, cross-breeds of the giant Belgian hare and the Lorraine rabbit were used; however, white New Zealand rabbits were used for strain 090R. These antisera were produced early at the University of Ulm for the survey (11) mentioned in the introduction. Antisera for group B types Ia, II, III, and the "wild strain" were produced here in Munich with Bayen rabbits.

Cultures. The cultures were received on blood-agar plates. One colony was transferred from each plate to a flask containing 40 ml of Todd-Hewitt broth (Difco) and incubated at 37°C for 14 to 16 hr. After addition of one drop of the sediment to 1 ml of sterile physiological saline for the immunofluorescence procedure, an extract of each culture for the precipitin test was prepared by the autoclave method of Rantz and Randall (8). This was found to be the most effective routine procedure for group B and group G strains (T. B. Smith and U. Wiemer, Arch. Hyg. in press). The precipitin tests were performed in capillary pipettes (15), and specific reactions were limited to those that occurred within 5 min (10).

Conjugation of immune globulin. The globulin frac-
tions of each antiserum were precipitated with sodium sulfate (14) and labeled with FITC by the method of Wood, Thompson, and Goldstein (17) as modified by Smith, Heymer, and Haferkamp (13). Essentially, 0.5 M carbonate-bicarbonate buffer (pH 9.5) was used in lieu of 0.1 M sodium hydroxide, and there was no further fractionation of the globulins after conjugation. In addition, 10 ml of group B streptococcal antiserum (control 548975) was purchased from Difco and labeled in the same manner. Fluorescein to protein ratios were determined on some of the conjugates.

Preparation and examination of smears. Smears of each culture were prepared and stained by the method described by Smith (10) and examined with a Leitz Ortholux fluorescence microscope with incident light. The assembly utilized during the study consisted of an Osram HBO-200 mercury vapor lamp, a Schott BG12 (3MM) exciter filter, a BG 38 blue filter, and a K530 barrier filter.

RESULTS

Response of rabbits to immunization. All of the rabbits developed good immune reactions within 1 month after the beginning of immunization. However, two rabbits, R IV, injected with strain 18RS (type II), and R VI, injected with strain M216 (type III), developed unusual precipitin reactions after only 3 weeks of immunization. The former, R IV, produced a heavy column of precipitate throughout the capillary pipette within 1 min; however, serum of the latter, R VI, gave no evidence of a precipitin reaction until 1 min after it had been mixed with the antigen extract and then precipitation proceeded slowly for 10 min. Further, after 1 hr the precipitate produced by the serum of R IV sank to a column 14 mm in height, whereas the precipitate produced by R VI sank to a column only 5 mm in height. There was no further change in either column at room temperature for 24 hr. In addition to this unusual reaction, all of the antisera produced by the strains from the British Central Public Health Laboratory gave type-specific reactions without absorption with heterologous types, with the exception of a weak reaction between anti-type 1b and type III precipitinogen (Table 1). On the other hand, the strain 090R (type Ia) antiserum produced strong precipitin reactions with all of the group B types studied (Table 1).

FITC-labeled globulins. Cross-reactions were observed with the labeled group A, C, and G immune globulins; the difficulty, however, was removed by absorption with heterologous streptococcal antigens (10). In contrast, the labeled group B immune globulins exhibited very little cross-reaction with groups A, C, and G and, except for type Ia (090R) and the “wild strain,” even less within the group. The type Ia immune globulin reacted with type II cells almost as strongly as with type Ia cells (Fig. 4), but the reaction with type 1b cells was moderate and with type III cells it was weak and mosaic-like (Fig. 2). This unexpected finding supplied a partial explanation of the difficulty that was encountered when only the labeled immune globulin produced by strain 090R was available.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Type Ia (090R) Precipitin</th>
<th>Fluorescence</th>
<th>Type Ib (H36B) Precipitin</th>
<th>Fluorescence</th>
<th>Type II (18RS) Precipitin</th>
<th>Fluorescence</th>
<th>Type III (M216) Precipitin</th>
<th>Fluorescence</th>
<th>Type II (wild) Precipitin</th>
<th>Fluorescence</th>
<th>Difco, fluorescence</th>
<th>Mixed conjugate, fluorescence</th>
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* Precipitating antiserum was not available.
On the basis of this information, a mixed conjugate, consisting of nine parts of type 1a and one part of type III, was prepared that was adequately reactive with all of the group B strains studied (Table 1).

Other than doubtful reactions, the labeled immune globulin produced from the Difco antiserum was reactive only with type 1b cells. In contrast with this, it is interesting that the "wild strain" was strongly reactive with type III cells as well as type II cells (Table 1) and that there was a weak reaction with types 1a and 1b.

The two antisera that were so strikingly different in their precipitation reactions (anti-type II and anti-type III) were equally different as labeled immune globulins, but the antibody activity was reversed. As far as the determination of the fluorescent to protein ratio can measure a conjugate, the two were practically equal. The type II conjugate contained a globulin concentration of 110.3 μg/ml and a fluorescent to protein ratio of 4.52, and the type III conjugate contained a globulin concentration of 109.9 μg/ml and a fluorescent to protein ratio of 4.50. Upon titrating the conjugates, however, the type II sample maintained a 4+ reaction until the concentration was reduced to 0.27 μg/ml (1:400 dilution), whereas the type III sample maintained a 4+ reaction until the concentration was reduced to 0.13 μg/ml (1:800 dilution).

Frequency of group B strains. Even though this study was not designed to determine the distribution of β-hemolytic streptococci in human infections, the isolation of group B strains from the cultures received, exclusive of urine sources, was too frequent not to be mentioned. This category constituted 82 of the 455 strains that were not isolated from urine, whereas there were only 55 isolates of group C and group G strains combined. Even more remarkable was the fact that the 82 group B strains were largely isolated from abscesses, diabetic ulcers, and cases of pneumonia and bronchitis.

DISCUSSION

The results obtained with the two antisera R IV and R VI strongly emphasize the fact that the fluorescent-antibody activity of an antiserum cannot be reliably predicted on the basis of the precipitating antibody activity (4, 13, 16). The difference probably results from the difference between the two reactions. For precipitation to occur, at least with rabbit immunoglobulin G, bivalent antibodies are required that have active sites complementary to the antigenic determinants (2). On the other hand, it is probable that any type of antibody binding, whether univalent or bivalent, is demonstrable with immunofluorescence. On this basis, it is probable that the R VI antiserum contained a significant quantity of antibodies to the group B type III antigen that did not precipitate antigen. Further, the light precipitate formed by R VI in comparison with the very heavy precipitation of R IV should not be interpreted as the result of the formation of soluble complexes, since, under the conditions of this experiment, soluble complexes are not formed in the zone of antibody excess (3, 9).

Of unusual interest is a comparison of the precipitin and immunofluorescence activities of the 090R antiserum (Table 1). This antiserum always produced moderately strong to very strong precipitation reactions with all group B strains; however, the fluorescent-antibody activity with types 1b and III was not satisfactory. Unlike the type 1b, type II, and type III antisera, it is obvious that the type 1a antiserum contained antibodies to the C polysaccharide of group B as well as the S polysaccharide. If this is correct, why was the immunofluorescent activity of the conjugate unsatisfactory with cells of types 1b and III? On the basis of the evidence available, it is probable that the S polysaccharide, a capsular antigen (7), interferes with binding of the group-specific antibodies with the C polysaccharide when the cell is intact. This difficulty is avoided, however, when the labeled conjugate contains antibodies to the S polysaccharide. It is most likely that the 090R (type 1a) conjugate contained a high concentration of cross-reacting antibodies to the type II S polysaccharide as well as type-specific antibodies. This point could be fully investigated with the use of S polysaccharide-free mutants of all of the major types of group B streptococci. Such strains, unfortunately, were not available to me during the study.

Figures 1 through 4 were photographed as indicated, without the smears being stained with the mixed conjugate. When a labeled conjugate contained an adequate concentration of antibodies to a given strain of group B streptococci, excellent fluorescence was obtained (Fig. 1 and 4). On the other hand, when the conjugate contained mainly cross-reacting antibodies in a reduced concentration, the result was the mosaic-like reaction illustrated by Fig. 2. Even though the fluorescence was weak, there is no difficulty in distinguishing this reaction from a negative reaction (Fig. 3). Although experience with the mosaic type of reaction that was produced by a significant number of group B strains in the presence of the type 1a conjugate enabled me to identify practically all of the group B strains that were received before the preparation of the mixed conjugate, the latter was found to be more
reliable due to the objectivity with which the results could be ascertained.

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LITERATURE CITED


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