Nontypable Group B Streptococci Isolated from Human Sources

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The present study was done to determine whether so-called nontypable (NT) group B streptococci from human sources possess as yet unrecognized type antigens. Antisera were raised in rabbits against several NT strains and then tested with hydrochloric acid extracts of 53 NT group B streptococci. One serum was strain specific, another was nonspecific in that it contained only R-protein antibodies, and a third (NT1), although apparently type specific, reacted with only five strains. These results do not justify using NT1 serum in the group B typing system.

Group B streptococci are divided into five serological types: Ia, Ib, Ic, II, and III (1, 2, 7). The antigens responsible for type specificity are both protein and polysaccharide and are related to the virulence of the organism (3). Some strains, however, appear to be devoid of type-specific antigens because hydrochloric acid extracts of the strains do not react with typing antisera currently used. Nontypable (NT) bovine strains were studied by British investigators, who concluded that these strains contained no type-specific antigens (5). The present study was done to see if NT strains isolated from human sources contain type antigens not yet described.

Vaccines of eight NT group B streptococci isolated from human sources (blood, urine, throat, vagina, wound, and exudate) were prepared as follows. One colony of each strain was picked to 30 ml of Todd-Hewitt broth, which was then incubated at 35°C overnight. The turbid broth was used as an inoculum for 400 ml of Todd-Hewitt broth, which was also incubated overnight at 35°C. The streptococcal cells were packed by centrifugation and then suspended in 75 ml of sterile physiological saline. The saline suspension was placed in a 60°C water bath for 45 min and was tested for purity before heating and sterility after heating by streaking blood agar plates. Rabbits were injected intravenously with 0.5 ml of vaccine for 3 successive days the first week and then with 1.0 ml for the same 3 successive days each subsequent week until tests of their sera showed strong type-specific antibody responses. The tests were done by precipitin (8) and Ouchterlony (4) analyses with hydrochloric acid extracts of the immunizing strains and of strains representing all five types. Type-specific responses to group B vaccines typically occur later than the group B-

specific response so that, in most of the sera, group B antibodies were either no longer detectable or present in only small amounts. Immunization of all rabbits was discontinued after 14 weeks.

The analyses of sera from rabbits immunized with NT group B streptococci led to the following conclusions. Three of the eight NT strains produced no type-specific antiserum. The remaining five strains, however, produced antisera that reacted strongly with extracts of the immunizing strain but that contained little or no group B antibodies. Ouchterlony studies with both untreated and pepsinized hydrochloric acid extracts showed that the specificity of three of these five antisera was against R-protein, a nonspecific streptococcal antigen (6), and that the other two antisera (NT1 and NT6) were specific for antigens that were probably polysaccharides. The three anti-R-protein sera were pooled for subsequent tests.

Extracts of all NT group B strains sent for identification to the Streptococcus Laboratory, Center for Disease Control, were tested by capillary precipitin tests with the anti-R-protein, NT1, and NT6 antisera. Of 53 such isolates, 21 gave positive precipitin reactions (Table 1). One serum (NT6) appeared strain specific because it reacted only with the NT6 immunizing strain.

Forty percent of the group B NT strains tested in this study gave positive capillary-precipitin reactions with antisera raised against several NT strains. The antisera that were used in the study contained no detectable antibodies against any of the type antigens currently recognized. However, one serum (NT6) was strain specific, another (anti-R-protein) was specific for R-protein antigens, which occur in several streptococcal groups, and the third (NT1) reacted with only five NT strains. Since

TABLE 1. Number of NT group B streptococcal strains reactive in the capillary-precipitin test with antisera raised against NT strains

Antiserum	No. of strains positive
R ^a	15
NT1	5
NT6	1

^a R, Anti-R-protein.

less than 3% of group B strains isolated from human sources are NT, it would not be useful at this time to use NT1 as part of the battery of group B typing antisera used in epidemiological studies.

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