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Human Fc(γ) Receptors for Differentiation in Throat Cultures of Group C "*Streptococcus equisimilis*" and Group C "*Streptococcus milleri*"

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The biochemical characteristics and the presence of human Fc(γ) receptors of 52 throat isolates of group C beta-hemolytic streptococci were examined. Among these isolates, 38 were identified as "*Streptococcus milleri*" and 14 were identified as "*Streptococcus equisimilis*." The differentiation of group C "*S. equisimilis*" from "*S. milleri*" with identical group antigens was easy to perform by the measurement of the size of the hemolytic zone on a sheep blood agar plate in an anaerobic atmosphere and by biochemical tests (Voges-Proskauer test). A clear-cut criterion for differentiation was noted among these isolates, i.e., the presence of Fc(γ) receptors. "*S. equisimilis*," which are generally associated with pharingitis, possess human Fc(γ) receptors, while "*S. milleri*," which are generally isolated from healthy persons, have no such receptors.

In conventional identification schemes, the streptococci are initially separated by their ability to hemolyse erythrocytes. Beta-hemolytic streptococci are subsequently identified by serological methods, whereas non-beta-hemolytic streptococci are differentiated to the species level by physiological tests (8, 10). The impact of serological studies of the streptococci by Lancefield has fostered reliance on antigenic characteristics as a major criterion of streptococcal taxonomy. Consequently, beta-hemolytic "*Streptococcus milleri*" strains with group A, C, or G antigen may be mistaken for other species of beta-hemolytic streptococci which have been traditionally associated with these Lancefield group antigens (i.e., *Streptococcus pyogenes*, group A; "*Streptococcus equisimilis*," group C in humans; and the large-colony group G streptococci [11, 12]).

The name "*S. milleri*" does not have nomenclatural standing because it is not on the Approved lists of bacterial names (19). Different studies (6, 7, 15) have indicated that strains of "*S. milleri*" are similar to minute-colony-forming streptococci of serological groups A, C, F, and G. In the Facklam system, minute beta-hemolytic strains are classified separately, by their Lancefield group antigens, as subtypes of *Streptococcus anginosus* (9).

Bucher and Von Graevenitz (3) have observed that the large-colony group C and G streptococci have been implicated as etiological agents of pharyngitis. However, they add that no convincing evidence exists for the pathogenic role of beta-hemolytic "*S. milleri*" in throat infections. In addition, Facklam (9) has indicated that the group A *S. anginosus* does not possess the same virulence factors possessed by the group A *S. pyogenes*.

In a preliminary study, we had noted that some strains of beta-hemolytic streptococci belonging to group A, C, and G possessed receptors for the Fc fragment of human immunoglobulin G (IgG) (14). But, at that stage, all of the beta-hemolytic streptococci were solely identified by their Lancefield group antigen. However, in a recent study reported by Ruoff et al. (18), "*S. milleri*" accounted for approximately 81% of 47 group C throat isolates.

These results led us to perform more accurate and sophisticated identifications of group C beta-hemolytic streptococci isolated from throat specimens. In this study, we have determined their physiological characteristics to differentiate the beta-hemolytic "*S. milleri*" group C (*S. anginosus* group C) from "*S. equisimilis*" group C. Furthermore, we have investigated the occurrence of Fc(γ) receptors on these group C streptococci, and we have noted their presence on "*S. equisimilis*" but never on "*S. milleri*."

MATERIALS AND METHODS

***Streptococcus* strains.** All strains tested were isolated from throat swabs of humans and were selected either from infected patients with symptoms of pharyngitis or from healthy medical students. The streptococci studied were beta-hemolytic isolates present in moderate to abundant amounts in cultures of throat specimens. Gram staining and the catalase tests were performed on all beta-hemolytic colonies to identify them presumptively as streptococci. All strains studied were beta-hemolytic on tryptic soy agar plates (Bio Merieux, Marcy L'Etoile, France) containing 5% sheep blood when incubated anaerobically at 35°C in a GasPak jar (Oxoid Biolyon, Lyon, France). Plates were examined for hemolysis and morphological colony size after 24, 48, and 72 h of incubation. These cultures were used to perform the tests described below.

Serological characterization. Beta-hemolytic streptococci were tested for the presence of Lancefield group antigen by both the Phadebact coagglutination tests (Pharmacia Diagnostics, Bois D'Arcy, France) and the Latex agglutination reagents (Wellcome Diagnostics, France) as described in the instructions of the manufacturers.

Identification by API 20 Strep. Biochemical testing was carried out by the API 20 Strep system (API System SA, Montalieu-Vercieu, France). The tests and interpretation criteria of tests are described in detail in the manufacturer's package insert and in other reports (1, 11, 17). Inocula for the API 20 Strep were prepared by growth on two sheep blood

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tryptic soy agar plates that had been incubated for 48 h in an anaerobic atmosphere; this method was especially useful for obtaining sufficient inocula (greater than or equal to a McFarland no. 4 turbidity standard) when testing small colonies.

Characterization of Fc(γ) receptors. Air-dried bacterial smears were fixed by dipping for 10 min in acetone at room temperature. As previously described (13), bacterial smears were treated with human Fc(γ) fragment, and then the binding of Fc(γ) on streptococci was revealed by immunofluorescence staining with fluorescein-labeled sheep $F(ab')_2$ fragments bearing an anti-Fc(γ) activity. The two steps of the reaction were performed at room temperature for 0.5 h. The tests were read on a Zeiss microscope equipped with a fluorescence vertical illuminator.

RESULTS

The 52 clinical isolates characterized in this study could be subdivided into large or typical colony size (0.5 to 1 mm) and pinpoint or atypical minute colony size. All of these strains were beta-hemolytic on sheep blood agar plates, but it must be noted that large variations in diameters of hemolytic zones were always observed in these subgroups. Among these isolates, all of the 14 large-colony group C streptococci grew normally in 24 h, and the diameters of hemolytic zones were always larger than 3 mm. All of the 38 minute colony group C streptococci grew slowly in 48 to 72 h, and the diameters of hemolytic zones were always smaller than 1 mm.

By use of the Rapid Strep system, 38 (small-colony forms) proved to be biochemically identical to "*S. milleri*." The remaining isolates included 14 "*S. equisimilis*" (large-colony forms). These latter strains required serogrouping to differentiate them from group G strains. Of the "*S. milleri*" isolates, 3 were identified as "*S. milleri*" biotype 1, 14 were identified as "*S. milleri*" biotype 2, and 21 were identified as "*S. milleri*" without distinction between biotype 1 or 2. These strains were differentiated on the bases of three physiological characteristics determined by the Rapid Strep system. All of the large-colony group C streptococci produced acid from ribose, hydrolyzed β glucuronic acid, and were negative for acetoin production from glucose (Voges-Proskauer [VP] reaction). In contrast, all of the small-colony isolates were negative for acid production from ribose, negative for β-glucuronidase, and positive in the VP test. The serogrouping was identical by both methods.

None of the group C "*S. milleri*" strains tested had receptors for the Fc(γ) part of human IgG, whereas all of the group C "*S. equisimilis*" strains had receptors, as detected by indirect immunofluorescence staining.

Of the 14 group C "*S. equisimilis*" strains, 13 were isolated from symptomatic carriers. The growth on primary plates always showed many colonies (more than 50) in pure culture or in association with saprophytic flora. On the contrary, of the 38 group C "*S. milleri*" strains, 26 were isolated from asymptomatic carriers. The growth on primary plates was always low (fewer than 10 colonies) or moderate (10 to 50 colonies) in association with saprophytic flora.

DISCUSSION

The results of this study indicate that Lancefield serogrouping does not provide sufficient information for species identification of beta-hemolytic group C streptococci isolated in throat specimens from humans. Our data suggest that a minimal number of morphological characteristics obtained on primary plates incubated in an anaerobic atmosphere could lead to misidentification of "*S. equisimilis*" group C and "*S. milleri*" group C. These isolates strains can be subdivided into large- and minute-colony forms on the basis of growth, size of colony, and, especially, diameter of hemolytic zone. For Lawrence et al. (12), the colony size was not a definite characteristic among these isolates, whereas for Ruoff et al. (18), all beta-hemolytic "*S. milleri*" isolates shared the common trait of small colony formation. These investigators (18) have also noted that some "*S. milleri*" isolates were identified as beta-hemolytic when the area around the subsurface growth was examined microscopically. However, those authors (12, 18) have not indicated that the hemolysis size could be a major morphological characteristic for differentiating the strains of "*S. equisimilis*" and "*S. milleri*" possessing the same group C antigen as it appears in our study. Isolation of our specimens on sheep agar plates and incubation in an anaerobic atmosphere could explain these differences, as was previously noted by Bucher and Von Graevenitz (3).

When the Rapid Strep system was used, all of the large-colony forms were biochemically identified as "*S. equisimilis*," and all minute-colony forms were identified as "*S. milleri*." Our data agree with the findings of Lawrence et al. (12), which were obtained by a different method. The Rapid Strep system is easy to perform but not very economical, and we think that the rapid VP test (3) could be sufficient to differentiate these streptococci in throat specimens.

Ruoff et al. (18) reported that 81% of 47 group C throat isolates were "*S. milleri*." For Lawrence et al. (12), "*S. milleri*" represented 56% of 48 clinical isolates of group C streptococci. As did Bucher and Von Graevenitz (3), we noted that typical strains of group C "*S. equisimilis*" are associated with pharyngitis, while the minute strains of group C "*S. milleri*" are generally isolated in healthy persons. However, the significance of these isolates in our study cannot be evaluated since concomitant viral cultures were not performed and anti-streptolysin O titers were not determined. The clinical significance of "*S. milleri*" has been recently reevaluated (20). However, in this study, 88% of the "*S. milleri*" strains were nonhemolytic, and most of the strains possessed F antigen. Nevertheless, "*S. milleri*" was considered of questionable significance in upper respiratory tract infections (20).

Indeed, we agree with most of the investigators (2, 11) that serogrouping is a more rapid, efficient, and useful technique for identifying most clinical isolates of beta-hemolytic streptococci. Nevertheless, serogrouping used solely on all beta-hemolytic strains without distinction of the size of hemolysis may lead to erroneous assumptions concerning the pathogenicity of the strains. Thus, we suggest that all minute colony group C streptococci isolated from throat specimens in an anaerobic atmosphere should be confirmed by a rapid VP test in a first approach to distinguish them from "*S. equisimilis*."

Although the term "*S. milleri*" is well established in European nomenclature (16), a different taxonomy for this group of organisms is used by Facklam (9). Nevertheless, our minute-colony-forming streptococci of beta-hemolytic group C "*S. milleri*" in the British taxonomy are similar to *S. anginosus* group C in the American taxonomy.

For the first time, therefore, we are displaying the presence of human Fc(γ) receptors only on large-colony forms of group C "*S. equisimilis*." In our previous study (14), we had noted that only 48% of 23 beta-hemolytic group C isolates

had Fc(γ) receptors. But at that time, all beta-hemolytic strains studied were identified solely by serogrouping and were not differentiated from "*S. milleri*." This misidentification led us to think that there was no relationship between the presence of human Fc(γ) receptors on streptococci and their pathogenicity in throat specimens (14). In the present study, in which we distinguished the different species of group C streptococci, the statistical results (χ^2 test) show that there is a significant relationship between the presence of Fc(γ) receptors on group C "*S. equisimilis*" and their origin from pharyngitis ($P < 0.001$). These surface structures on some group A, C, and G strains with a high affinity for immunoglobulin might contribute to pathogenicity. A link between the presence of IgG Fc(γ) receptors and virulence was described recently by Burova et al. (5). Furthermore, those authors have shown that the presence of IgG Fc receptors inhibits phagocytosis of streptococci in classical bactericidal tests, probably through interference with antibody-dependent complement activation (4). In addition, Facklam (9) has indicated that the minute-colony-size group A streptococci do not possess the antiphagocytic factors (M protein) possessed by typical group A streptococci (*S. pyogenes*), as indicated by in vitro bactericidal tests.

This study demonstrates that correct identification of beta-hemolytic group C streptococci isolated from humans can be achieved by use of the combination of morphology on sheep blood agar plates, especially the diameter of the hemolytic zone in an anaerobic atmosphere, and the VP reaction. We have especially noted that group C "*S. equisimilis*," generally associated with pharyngitis, possess receptors for the fragment Fc(γ) of IgG, while group C "*S. milleri*," generally isolated from healthy persons, have no receptors. We are continuing to study the virulence of these different group C streptococci isolated from throat specimens.

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