Differentiation of species in human β-haemolytic group G streptococci using immunoglobulin Fc fragment receptor

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Abstract

Aims: To assess the ability of human immunoglobulin Fc fragment binding activity to differentiate human biotype large colony group G streptococci from the group G "Streptococcus milleri group".

Methods: Fifty two isolates of large colony group G streptococci and 30 group G "S milleri group" strains were tested for their ability to bind fluorescein conjugated human IgG Fc fragments after acetone fixation. Immunoblotting with peroxidase labelled human Fc fragments after resolution of bacterial polypeptides by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for six large colony strains.

Results: All large colony group G streptococci showed positive Fc fragment binding whereas all "S milleri group" bacteria failed to bind Fc fragments when viewed by fluorescence microscopy. All six large colony strains showed similar immunoblot binding patterns.

Conclusion: Immunoglobulin Fc fragment receptor content distinguishes the large colony group G streptococci from the group G "S milleri group" and may have a role in the rapid laboratory diagnosis of pharyngeal pathogens.

The potential of β-haemolytic Lancefield group G streptococci to cause serious disease is not doubted, but their status in streptococcal taxonomy is less clear. β haemolytic group G streptococci have been differentiated into small and large colony phenotypes.1 The small colony strains are collectively termed “Streptococcus milleri group,” synonymous with Streptococcus anginosus, Streptococcus milleri, and Streptococcus anginosus-milleri group,” although genetic heterogeneity has been suggested.2 Large colony forms may be biotyped to facilitate recognition of human rather than animal strains.3 Furthermore, human source large colony group G streptococci are highly related to S equisimilis (group C) and some group L streptococci.4

Lebrun et al initially proposed that immunoglobulin Fc receptors were present in some group C and G streptococci, but subsequently amended their observations for group C streptococci by showing that S equisimilis rather than group C “S milleri group" had such receptors.5 This trait served consistently to separate these two subsets of group C streptococci.6

Given the similarity of large colony forms of group G streptococci with S equisimilis, and the recognition that some group G streptococci have immunoglobulin binding activity,7 we sought to determine whether Fc receptor state would differentiate the large from small colony forms of group G streptococci.

Methods

Eighty two strains of β-haemolytic group G streptococcal isolates were obtained from the pharynges of children from 1988 to 1989 inclusive. Fifty two of these isolates were classified as large colony human biotype strains on the basis of a negative rapid Vogues-Proskauer (VP) test, sugar fermentation (lactose, raffinose, sorbitol, trehalose), and aesculine hydrolysis.8 Thirty group G strains were classified as "S milleri group" based on quality of haemolysis on sheep blood agar (Columbia base), colony size, and a positive rapid VP test. Isolates were stored at −70°C.

FC FRAGMENT BINDING ASSAY

Bacteria were subcultured once on to blood agar plates at 37°C in 5% carbon dioxide overnight. Suspensions of each strain were prepared in phosphate buffered saline (PBS) (pH 7.2) to approximate a 0.5 McFarland turbidity standard. Each suspension (10 μl) was dried on to preformed microscope wells at room temperature and fixed with cold acetone for five minutes. A 1 in 100 dilution of a fluorescein conjugated human IgG Fc fragment (Jackson Immunoresearch Laboratories, West Grove, Pennsylvania in PBS was added to cover the bacteria in each well. Slides were incubated at 37°C for 30 minutes in a humidified chamber and then washed in PBS five times. After drying at room temperature each slide was mounted with Bacto FA mounting fluid (Difco, Detroit, Michigan) and a coverslip and viewed under fluorescence illumination.

FC FRAGMENT IMMUNOBLOTTING

Bacteria were cultured in a Todd-Hewitt broth overnight at 37°C, centrifuged to form a pellet, and washed with PBS. Whole bacterial cells were boiled in a reducing sample buffer and the resulting material was resolved by SDS-PAGE. After the same resolution an
Differentiation in human \(\beta\)-haemolytic streptococci

The observed homogeneity of Fc binding ability among large colony group G streptococci is another feature that these bacteria have in common with \(S\) equisimilis. We have previously shown phenotypic similarity among the \(\beta\)-haemolytic group \(C\) and \(G\) streptococci are frequently isolated from both asymptomatic and symptomatic (pharyngitis) subjects, we have proposed an association between the isolation of \(S\) equisimilis and large colony group \(G\) streptococci with symptoms. In contrast, strains with Lancefield group \(C\) and \(G\) antigens of the “\(S\) milleri group” were not associated with disease. An approach to easily and rapidly differentiating these subsets of the \(\beta\)-haemolytic non-group \(A\) streptococci in the microbiology service laboratory could then be useful, although the experienced technologist will often be able to differentiate these subsets by visual inspection of subcultures.

Although the rapid VP test has been proposed for rapid differentiation we have found the assay to be more inoculum dependent than \(\beta\)-D-glucuronidase activity determination. The fluorescence Fc fragment binding assay described here has the potential to be even less inoculum dependent than the latter two methods, because in theory even less micro-organism may be required to determine a positive test. A scheme of Lancefield grouping followed by either \(\beta\)-D-glucuronide conjugate hydrolysis or Fc-FITC binding may then expedite the laboratory diagnosis of an infecting pathogen.

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