

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

N Cimolai, A C H Cheong

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,^{7,8} 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 aesculin hydrolysis.^{3,9} 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

Division of Medical Microbiology, Department of Pathology, University of British Columbia
N Cimolai
A C H Cheong

Correspondence to:
Dr N Cimolai, Program of Microbiology, Department of Pathology, British Columbia's Children's Hospital, Vancouver, British Columbia, Canada V6H 3V4.

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electrophoretic transfer to nitrocellulose was performed. Transblots were then blocked overnight at 4°C in 1% skimmed milk-PBS. On the following day they were washed in PBS and then reblocked with 0.25% gelatin/0.25% Tween 20-PBS for one hour at 37°C. After an additional PBS wash a 1 in 800 dilution of peroxidase labelled-human Fc fragments (Jackson Immunoresearch Laboratories) in PBS was added and incubated at 37°C for one hour. After a final wash 4-chloronaphthol enzyme substrate was used.

Results

The Fc fragment binding assay clearly differentiates among the two large subsets of group G streptococci. All large colony human biotype strains of group G streptococci were positively fluorescent. This implies that these bacteria can uniformly bind human immunoglobulin Fc fragments. In contrast, all group G streptococci of the "*S. milleri* group" lacked the ability to bind Fc fragments. The appearance of fluorescent cells was similar to that described for Fc binding group C streptococci.^{5,6} The presence of fluorescence, and therefore Fc binding, could be established microscopically with scanning at $\times 100$ magnification, and confirmation could be obtained at $\times 1000$ magnification by viewing individual cells.

A random sampling of six large colony strains was evaluated by Fc fragment immunoblotting. All six strains showed similar binding patterns: Fc-peroxidase was bound to antigens with migration rates that approximate 29 and 55 kilodaltons. We have found similar Fc binding antigens in *S. equisimilis* but not "*S. milleri* group" (group C and G) strains when the bacterial cells have been processed in a similar manner.

Discussion

The ability of some group G streptococci to bind human immunoglobulins is not new.⁷ Understanding in the molecular biology of binding specificities and receptors has progressed considerably over the past two decades,¹⁰ and technology transfer has led to the exploitation of these immunoglobulin binding proteins, such as protein G, in a manner analogous to staphylococcal protein A. The approximate molecular weights of these binding proteins as we have defined them by immunoblotting differ from those of some previous reports, but we attribute such differences to the variation in which the binding proteins are extracted and solubilised—for example, various enzymatic hydrolyses, heat treatment, acid treatment.^{8,10} Such variation has led to the demonstration of binding proteins with approximate molecular weights ranging from 38 to 69 kilodaltons. Among the numerous immunoglobulin binding proteins thus far recognised in an array of bacterial species, the Fc-binding proteins common to the group C and group G streptococci have been designated type III Fc receptors. Such receptors are distinguished from other Fc receptors on group G streptococci of animal origin.¹¹

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 these bacteria by an assessment of certain enzyme substrate profiles,¹² and Efstratiou has shown the ability of the same organisms to share T type proteins.¹³ All of these phenotypic similarities reflect homology which has been previously shown at the genetic level.⁴

Apart from illustrating phenotypic similarity, the finding of Fc fragment binding activity among group G streptococci has the potential to contribute to the rapid laboratory diagnosis of putative pharyngeal pathogens. Although β -haemolytic group C and G streptococci are frequently isolated from both asymptomatic and symptomatic (pharyngitis) subjects,¹ we have proposed an association between the isolation of moderate to heavy quantities of *S. equisimilis* and large colony group G streptococci with symptoms.¹⁴ 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 β -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 differentiation⁹ we have found the assay to be more inoculum dependent than β -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 β -D-glucuronide conjugate hydrolysis or Fc-FITC binding may then expedite the laboratory diagnosis of an infecting pathogen.

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- 1 Cimolai N, Elford RW, Bryan L, Anand C, Berger P. Do the beta-hemolytic non-group A streptococci cause pharyngitis? *Rev Infect Dis* 1988;10:587-601.
- 2 Whitley RA, Hardie JM. DNA-DNA hybridization studies and phenotypic characteristics of strains within the "*Streptococcus milleri* group". *J Gen Microbiol* 1989; 135:2623-33.
- 3 Clark RB, Berrafati JF, Janda JM, Bottone ET. Biotyping and exoenzyme profiling as an aid in the differentiation of human from bovine group G streptococci. *J Clin Microbiol* 1984;20:706-10.
- 4 Farrow JAE, Collins MD. Taxonomic studies on streptococci of serologic groups C, G, and L and possibly related taxa. *Systematic Applied Microbiology* 1984;5: 483-93.
- 5 Lebrun L, Pillot J, Grangeot-Keros L, Rannou M. Detection of human Fc receptors on streptococci by indirect immunofluorescence staining: a survey of streptococci freshly isolated from patients. *J Clin Microbiol* 1982;16:200-1.
- 6 Lebrun L, Guibert M, Wallet P, DeManeville M, Pillot J. Human Fc receptors for differentiation in throat cultures of group C *Streptococcus equisimilis* and group C "*Streptococcus milleri*". *J Clin Microbiol* 1986;24:705-7.
- 7 Kronvall G. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin G. *J Immunol* 1973;111:1401-6.
- 8 Reis KJ, Hansen HF, Bjorck L. Extraction and characterization of IgG Fc receptors from group C and group

- G streptococci. *Mol Immunol* 1986;23:425-31.
- 9 Lawrence J, Yajko DM, Hadley WK. Incidence and characterization of beta-hemolytic *Streptococcus milleri* and differentiation from *S pyogenes* (group A), *S equisimilis* (group C), and large-colony group G streptococci. *J Clin Microbiol* 1985;22:772-7.
 - 10 Boyle MDP, ed. *Bacterial immunoglobulin-binding proteins: Microbiology, chemistry, and biology*. New York: Academic Press, 1990.
 - 11 Myhre EB, Holmberg D, Kronvall G. Immunoglobulin-binding structure on bovine group G streptococci different from Type III Fc receptors on human group G streptococci. *Infect Immun* 1979;23:1-7.
 - 12 Cimolai N, Mah D. β -D-Glucuronidase activity assay for rapid differentiation of species within β -haemolytic group C and G streptococci. *J Clin Pathol* 1991;44:824-5.
 - 13 Efstratiou A. Outbreaks of human infection caused by pyogenic streptococci of Lancefield groups C and G. *J Med Microbiol* 1989;29:207-19.
 - 14 Cimolai N, Morrison BJ, MacCulloch L, Smith DF, Hlady J. Beta-hemolytic non-group A streptococci and pharyngitis: a case-control study. *Eur J Pediatr* 1991;150:776-80.