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# NOTES

## Analysis of Immunoglobulin G-Binding-Protein Expression by Invasive Isolates of *Streptococcus pyogenes*

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**Invasive group A streptococcal isolates collected as part of a Centers for Disease Control and Prevention surveillance study were analyzed for expression of immunoglobulin G (IgG)-binding proteins. Two IgG-binding phenotypes of group A isolates of the M1 serotype were identified. The first group expressed a surface protein that bound all four human IgG subclasses (type IIo) and was recognized by rabbit anti-serotype M1-specific antiserum but not by normal rabbit serum. The second group expressed an IgG-binding protein that was also recognized by the anti-serotype M1 antiserum but demonstrated significant nonimmune reactivity only with human IgG3 (type IIb). Analysis of extracts of the isolates for reactivity with human IgA, fibrinogen, and albumin was also performed. The importance of the binding of human plasma proteins to pathogenic group A streptococci remains to be established.**

Group A streptococci cause a variety of human diseases ranging from mild pharyngeal infections to severe invasive disease and the postinfection sequelae of rheumatic fever and glomerulonephritis (3, 4). The number and severity of group A streptococcal infections decreased in the postantibiotic era, but in the mid-1980s a resurgence of severe group A streptococcal disease, including a previously unknown streptococcal toxic-shock-like syndrome, occurred worldwide in immunocompetent patients with no known predisposing factors (6, 9, 10, 13, 19, 20).

A detailed analysis of group A strains collected as part of a Centers for Disease Control and Prevention (CDC) (Atlanta, Ga.) surveillance study of the recent resurgence of severe group A streptococcal infections and a toxic-shock-like syndrome has recently been reported (20). These studies determined a wide range of genotypic and phenotypic properties of these organisms. The phenotypic characteristics included the M protein type, serum opacity factor production, and production of pyrogenic exotoxins A and B as well as production of protease activity. At the gene level the presence of *speA*, *speB*, and *speC* genes was also determined. Type M1 isolates were found to predominate, and an association between this serotype and protease production was noted (20). Protease-producing isolates were found to be more frequently associated with soft tissue necrosis. The production of SpeA was significantly associated with rashes, shock, and organ involvement. Despite these associations, there was no single characteristic of group A streptococci that correlated directly with streptococcal toxic-shock-like syndrome. The isolates from that study were not assessed for expression of different forms of immunoglobulin-binding protein(s).

The present study was designed to characterize the immunoglobulin G (IgG)-binding-protein expression of representative isolates from the recent CDC surveillance study to determine if an epidemiological association between IgG-binding-protein expression and organisms causing severe streptococcal disease could be documented.

Thirty-three of the 62 strains originally characterized by the CDC as part of their surveillance study were selected for analysis. The characterization of these organisms provided by the CDC and originally reported by Talkington et al. (20) are summarized in Table 1. The isolates were obtained from the CDC as glycerol stocks and had not been passaged in the laboratory on more than three occasions. All strains used in these studies were grown from the original −70°C glycerol stock.

In the initial studies, the selected group A streptococcal isolates were analyzed for the presence of *fcrA* genes, class I or II *emm* or *emm*-like genes, and *enn* genes by PCR methods by using the gene-selective primers and conditions described previously by Podbielski and colleagues (14–16). The results of these studies are presented in Table 2. Twenty-three of the 33 strains contained an *emm*(-like) class I gene sequence and 11 contained an *emm*(-like) class II gene sequence as determined by amplification of a defined chromosomal DNA sequence with specific primers previously described (15). The chromosomal DNA from one strain demonstrated hybrid characteristics and was amplified with both the class I and class II *emm* (-like)-selective primers. Twelve of the 33 strains contained an *fcrA* gene, and, with one exception, this was present only in isolates containing a PCR-amplifiable *emm*(-like) class II gene. Eleven of the 33 isolates contained an *enn* gene, and all of the these isolates also contained an *fcrA* gene and an *emm*(-like) class II gene (Table 2).

In order to determine the expression of the various gene products by these isolates, the functional and antigenic profiles of the IgG-binding proteins in CNBr extracts of these isolates were determined by Western immunoblotting techniques by the procedures previously developed by our laboratory (17).

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TABLE 1. Profile of group A streptococcal isolates analyzed[a]

| M protein serotype | No. of isolates | No. of isolates[b] | | No. of isolates positive for: | | | | |
|---|---|---|---|---|---|---|---|---|
| | | SOR$^+$ | SOR$^-$ | Toxic shock | Toxin A | Toxin B | Both toxin A and toxin B | Neither toxin A nor toxin B |
| Nontypeable | 15 | 10 | 5 | 7 | 3 | 9 | 2 | 5 |
| M3 | 3 | 1 | 2 | 2 | 2 | 2 | 2 | 1 |
| M1 | 15 | 0 | 15 | 11 | 9 | 10 | 6 | 2 |
| Total | 33 | 11 | 22 | 20 | 14 | 21 | 10 | 8 |

[a] Data on these isolates were provided by the CDC and are detailed in reference 20. The mean age of patients from which isolates were obtained was 53.6 years.
[b] The serum opacity reaction (SOR) was scored as positive (+) or negative (−) (20).

The results summarized in Table 2 demonstrate that all 33 strains expressed IgG-binding proteins, although differences in the functional and/or antigenic profiles were observed. All 23 strains whose chromosomal DNA contained an *emm*(-like) class I gene expressed an immunoglobulin-binding protein reactive with the anti-type IIo antibody probe. Of these isolates, 7 expressed an IgG-binding protein reactive with all four human IgG subclasses (type IIo), while 15 of the isolates expressed an IgG-binding protein which reacted preferentially with human IgG3 (type IIb). Of the 12 strains containing an *fcrA* gene, 11 expressed a protein that was recognized by the anti-FcrA antibody probe. In general, those strains containing an *fcrA* gene expressed a type IIa immunoglobulin-binding protein reactive with human IgG1, IgG2, and IgG4. Three of the 12 *fcrA*-positive isolates also expressed an additional, antigenically unrelated protein which bound only human IgG3 (Table 2).

A number of recent studies have suggested that in addition to binding immunoglobulins, certain group A streptococcal M or M-like proteins have the ability to bind to other serum proteins, in particular, albumin and fibrinogen (5, 18) or human IgA (2, 3, 11, 12), which might contribute to their pathogenic potential. Albumin and fibrinogen binding is usually associated with *emm* or *emm*(-like) gene products, while IgA binding has generally been associated with the *enn* gene product. The reactivity profiles of cyanogen bromide extracts of each isolate were further examined for human albumin, fibrinogen, or IgA binding by Western blotting techniques as described previously (5, 17). The results of these studies are presented in Table 3.

Cyanogen bromide extracts of 32 of the 33 isolates contained fibrinogen-binding proteins. These fibrinogen-binding proteins corresponded to the same band as that which reacted with human IgG subclasses. Approximately two-thirds of the CNBr extracts contained proteins that bound human albumin to the same band as that reactive with human IgG. By contrast, extracts from less than 15% of the strains were found to react with human IgA (Table 3). The IgA-binding protein bands were distinct from those reactive with human IgG. All of the IgA-binding extracts were derived from strains which contained an *enn* gene; however, not all CNBr extracts of *enn*-positive isolates contained an extractable IgA-binding protein. Previous studies have suggested that *enn* gene expression occurs at a low level and that the gene product may not be expressed on the cell surface in high concentrations under normal growth conditions (3). Analysis of recombinant IgA-binding *enn* gene products has indicated that IgA-binding activity is retained following treatment with CNBr (unpublished observation).

Analysis of the distribution and expression of different functional or antigenic immunoglobulin-binding proteins and analysis of the binding of other plasma proteins did not identify a single plasma protein-binding property that associated with strains isolated from patients with toxic-shock-like syndrome. The most notable feature of this analysis was the appearance of two distinct IgG-binding-protein reactivity profiles among the 15 group A isolates of the M1 serotype (Table 2). This was of particular interest in view of recent reports that have identified the M1 protein itself as an IgG-binding molecule (18) and which have described the existence of a related IgG-binding-protein gene (protein H) in the *vir* regulons of certain M1 isolates (1, 7, 8). We have analyzed in

TABLE 2. Profile of IgG-binding proteins and genotypes of selected group A isolates

| M protein serotype | No. of isolates | No. with gene profile by PCR | | | | No. with IgG-binding-protein profile | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Functional[a] | | | Antigenic[b] | |
| | | *emm*(-like) I | *emm*(-like) II | *fcrA* | *enn* | Type IIo | Type IIa | Type IIb | Anti-FcrA | Anti-type IIo |
| M1 | 15 | 15 | 0 | 0 | 0 | 6 | 0 | 9 | 0 | 15 |
| M3 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 3 |
| Nontypeable | 15 | 0 | 10 | 10 | 10 | 0 | 10 | 3 | 10 | 0 |
| | | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| | | 4 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 4 |
| Total | 33 | 23 | 11 | 12 | 11 | 7 | 11 | 18 | 11 | 23 |

[a] The designation of functional activities follows that previously reported (17).
[b] These antibody probes recognize the two major antigenic families of type II IgG-binding proteins (17).

TABLE 3. Reactivities of CNBr extracts of selected group A streptococci with other, non-IgG, human plasma proteins[a]

| M protein serotype | No. of isolates | No. of extracts reactive with human: | | |
|---|---|---|---|---|
| | | Albumin | Fibrinogen | IgA |
| M1 | 15 | 12 | 15 | 0 |
| M3 | 3 | 3 | 3 | 0 |
| Nontypeable | 15 | 7 | 14 | 4 |
| Total | 33 | 22 | 32 | 4 |

[a] Determined by Western blotting techniques as previously described (5, 17).

detail these M1 isolates to determine whether the difference in IgG-binding profile is a consequence of expression of more than one gene product or of a modification of the M1 gene itself (16a). These studies may help to explain the different patterns of disease caused by serotype M1 isolates of group A streptococci.

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