

## **Binding of IgA and/or IgG is a common property among clinical isolates of group A streptococci**

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### SUMMARY

Certain strains of group A streptococci are known to bind IgA and/or IgG via a cell surface receptor, which may act as a virulence factor. The distribution of such receptors among routine clinical isolates was studied, using a total of 225 strains and an assay based on the binding of radiolabelled immunoglobulins. Among 194 throat strains isolated during three different time periods in two different geographical areas of Sweden, 82% showed significant binding of IgA and/or IgG. Studies on 31 septicaemia strains, isolated over a period of more than 8 years, showed binding for 84% of the isolates. The binding strains were of several different T-types and could be subdivided into two groups, those binding both IgA and IgG and those binding IgG only. These data show that binding of IgA and/or IgG is a very common property among clinical isolates of group A streptococci.

### INTRODUCTION

The recent outbreaks of rheumatic fever in the United States [1], and the reappearance of severe infections associated with a toxic shock-like syndrome [2, 3], have caused a renewed interest in infections caused by group A streptococci and in the extracellular proteins produced by these bacteria. Among such proteins, particular attention has been given to the scarlet fever toxins as well as to the M proteins, the antiphagocytic fibrils extending from the bacterial cell surface [4]. In addition to these well-studied proteins, some strains of group A streptococci are also known to express a cell-surface receptor with affinity for the Fc part of IgA or IgG [5–8]. The structure and binding properties of such immunoglobulin receptors has now been studied in considerable detail, but it has not yet been possible to define their biological role, although the available evidence suggests that they are virulence factors, like the M proteins [9, 10]. Information concerning the expression of the immunoglobulin receptors by routine clinical isolates of group A streptococci is also limited. There is evidence that receptors which bind IgG are common [11, 12], but only little is known concerning the distribution of receptors that preferentially bind IgA. However, the expression of IgA receptors is of particular interest, since most infections with group A streptococci occur on mucous membranes, where IgA is the predominating antibody. These con-

siderations, and the changing clinical situation, have prompted us to study a large number of routine isolates of group A streptococci for ability to bind IgA and IgG. The isolates studied included both throat strains and septicaemia strains, and a sensitive method was used to test these strains for the ability to bind immunoglobulins.

#### MATERIALS AND METHODS

##### *Bacterial strains*

The 194 throat strains of group A streptococci studied here were isolated from routine clinical specimens sent to either of two clinical microbiology laboratories. The strains were collected during three different time periods and in two different geographical areas of Sweden, and care was taken to avoid more than one isolate from local outbreaks or from the same family. The first 64 strains, series I, were isolated at Lund University Hospital in southern Sweden during February 1987. Series II, 50 strains, were collected in January 1988 at Karolinska Hospital in Stockholm, 600 kilometres north of Lund. Series III, 80 strains, were isolated in Lund in September 1989. All of these strains were sent to the two laboratories from different out-patient clinics in the surrounding counties.

The 31 septicemia strains of group A streptococci were isolated from blood cultures, at the Lund laboratory, during the period January 1981 to October 1989.

Following primary isolation, all strains were immediately frozen at  $-80^{\circ}$  in foetal calf serum. For binding tests, new plates were streaked from the frozen tubes and cultures in Todd-Hewitt broth were started from these plates. For one series of strains, binding tests were also performed with cultures started directly from the original plates. The results were very similar to those obtained later with cultures started from frozen samples of the same strains, showing that the freezing did not affect the binding ability of the strains.

All strains studied here were classified as group A streptococci with standard bacteriological techniques and were bacitracin-sensitive.

##### *Immunoglobulins*

Polyclonal human serum IgA was purchased from Cappel-Organon Teknika, Turnhout, Belgium, and polyclonal human IgG was purchased from Sigma Chemical Co.

##### *Binding assays*

Bacterial strains were tested for ability to bind radiolabelled IgA or IgG, as previously described [13]. Briefly, overnight cultures in Todd-Hewitt broth were washed and resuspended in PBSAT (0.12 M NaCl, 0.03 M phosphate, 0.02%  $\text{NaN}_3$ , 0.05% Tween 20; pH 7.2) to an absorbancy corresponding to about  $10^9$  bacteria per ml. Duplicate samples containing 2–5 ng (about  $10^4$  cpm) of radiolabelled protein in 25  $\mu\text{l}$  PBSAT were mixed with 200  $\mu\text{l}$  of bacterial suspension and incubated for 60 min at room temperature in plastic tubes. Two ml of PBSAT was then added, the tubes were centrifuged and radioactivity in the pellets was measured. The quantity bound is expressed as a percentage of added radioactivity. Non-specific uptake (less than 5%) recorded with a non-binding strain of *Staphylococcus epidermidis* has been subtracted.

*T-typing*

Classification of streptococcal strains according to T-type was performed with antisera purchased from the Institute of Sera and Vaccine, Prague, Czechoslovakia, following the instruction of the supplier. Briefly, a 2 ml overnight culture in Todd-Hewitt broth was centrifuged, and the pellet was resuspended in 0.5 ml of 0.15 M Tris buffer, pH 7.4. After addition of 60  $\mu$ l of trypsin solution (5% in water), the suspension was incubated at 37° for 30 min and then used for agglutination tests at room temperature. One drop of the trypsin digested bacteria and one drop of antiserum were mixed on a glass slide, followed by gentle rocking. The slide was inspected for agglutination within 2 min. Antisera corresponding to the following T-types were used: 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 22, 23, 25, 27, 28, 44, B3264 and Imp. 19. No attempt was made to classify the strains according to M-type.

## RESULTS

*Binding of IgA and IgG to throat strains*

The throat strains studied comprised three different series with 64, 50, and 80 strains, respectively. These strains were isolated in two different geographical areas of Sweden and during three different time periods. Data for all three series of strains are presented in Fig. 1, which shows that there is great variation in binding ability between individual strains. This variation is not due to the method used to measure binding, since repeated testing of several strains showed good reproducibility. For the analysis of these data, we have defined significant binding as > 8% binding. This cut-off value was chosen, since retesting of several different bacterial strains indicated that any binding above 8% was indeed significant (data not shown). Using this cut-off value, one can subdivide the throat strains that bind immunoglobulin into two groups, those that bind both IgA and IgG and those that bind IgG only. Surprisingly, binding of IgA alone was not observed for any of the strains. Among all these throat strains, 159/194 (82%) showed significant binding of IgA and/or IgG. Classification of all strains with regard to T-type (Table 1), showed that the binding strains were of several different types, in particular T-types 1, 4, 12, and 28. During the time period (2 years and 8 months) between the isolation of the strains in series I and those in series III, there was an increase in the frequency of IgG-binding strains of type T1 and a decrease in the frequency of non-binding strains, which were of type T12 or nontypable (data not shown). As a result, the percentage of binding strains increased from 63% in series I to 96% in series III. However, also in series III most of the binding strains were of T-types other than T1.

*Binding to septicaemia strains*

All septicaemia strains of group A streptococci isolated in our laboratory during a period of more than 8 years were tested for binding. Among these strains, 84% (26/31) showed significant binding of IgA and/or IgG. The variation between different strains was similar to that observed for the throat isolates (data not shown). Like the throat strains, the binding septicaemia strains could be

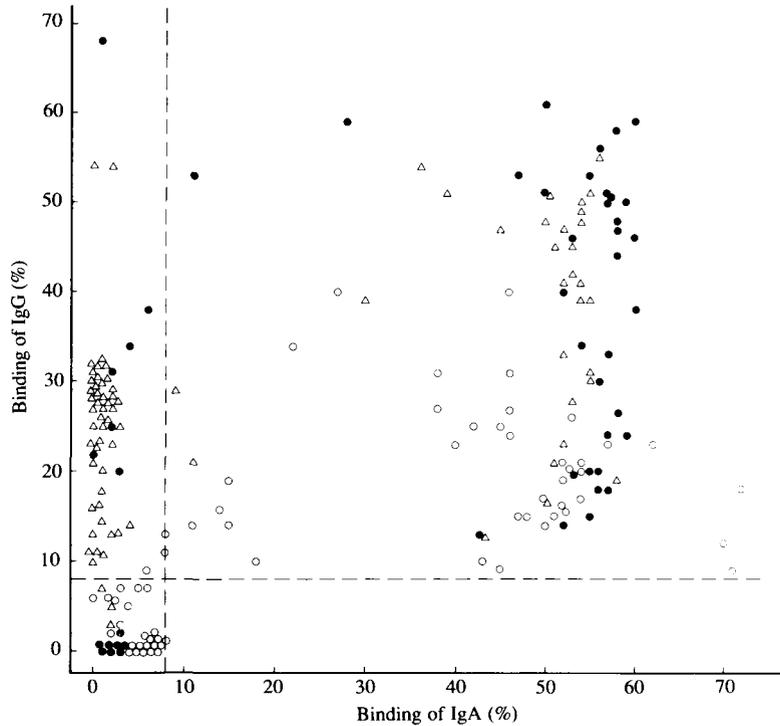


Fig. 1. Binding of IgA and IgG to 194 throat isolates of group A streptococci. Each symbol represents one strain. The different symbols correspond to the three series of strains mentioned in the text: Series I (○); Series II (●); Series III (△). The dashed lines indicate the position of the cut-off values used to subdivide the strains into binders and non-binders.

subdivided into two groups, those that bind both IgA and IgG and those that bind IgG only, and these strains were of several different T-types (Table 1).

Among the 31 septicaemia strains studied here, seven were isolated during a 7-month period from October 1988 to April 1989, and all of these strains were of type T1. These data are in agreement with reports that septicaemias and other severe infections due to group A streptococci type T1 have recently increased in frequency in both Great Britain and Scandinavia [3, 14]. Most of these T1 strains were IgG-binders, but the binding observed was of low magnitude (about 20% in the type of test used for Fig. 1).

#### DISCUSSION

The data presented in this paper show that binding of IgA and/or IgG is a common property among clinical isolates of group A streptococci in Sweden. Among a total of 225 strains, isolated from throat or blood cultures, 82% (185/225) were able to bind immunoglobulins. This high frequency cannot be explained by temporary accumulation of one type of binding strain in the population, since several different series of strains, isolated during different periods of time, gave similar results. In addition, the binding strains were found to be of several different T-types.

Table 1. Group A streptococcal strains from clinical specimens: classification according to immunoglobulin binding and T-type

T-Type	Throat strains that bind (n = 194)			Septicemia strains that bind (n = 31)		
	IgA and IgG	IgG	None	IgA and IgG	IgG	None
1	—	34	—	1	8	—
2	3	1	—	—	—	—
4	18	1	—	1	2	—
6	1	—	—	2	—	—
11	4	—	—	—	—	—
12	13	9	22	—	—	3
13	1	—	—	—	1	—
23	—	—	—	1	—	—
25	3	—	—	—	—	—
27	1	—	—	1	—	—
28	39	1	—	3	1	—
3,13,B3264	—	1	—	—	—	—
5,13,B3264	1	—	—	—	—	—
NT*	18	10	13	2	3	2
Subtotal: no. (%)	102(53)	57(29)	35(18)	11(36)	15(48)	5(16)

\* NT, nontypable.

As shown in Fig. 1, the immunoglobulin-binding strains studied here can be divided into two subgroups, those binding both IgA and IgG and those binding IgG only. Binding of IgA alone was not observed for any of the strains. These findings are in good agreement with the known properties of purified immunoglobulin receptors from group A streptococci. Thus, two variants of the IgA receptor, called protein Arp, were also shown to exhibit a weak binding of IgG [13, 15]. Strains expressing these receptors showed about 60% binding of IgA and 20% binding of IgG in the type of binding test used for Fig. 1. On the other hand, receptors that bind only IgG have been purified from strains of group A streptococci which bind only this immunoglobulin class [7, 16, 17]. However, the data in Fig. 1 show that some strains bind both IgA and IgG well. Several of these strains showed 50% binding, or more, for both immunoglobulins. A simple explanation for the binding properties of such strains could be that they express both receptors described above, but biochemical characterization has indicated that at least some of these strains express a new type of immunoglobulin receptor, which binds both IgA and IgG with high affinity (Lindahl and Stenberg, in preparation). This suggests that the immunoglobulin receptors in group A streptococci can be subdivided into at least three different groups: (a) those that bind IgA well and also exhibit a weak binding of IgG; (b) those that bind both IgA and IgG well, and (c) those that bind IgG only. It seems possible that further subdivisions will be possible in the future, when more is known about the structure and binding properties of immunoglobulin receptors isolated from many different streptococcal strains.

The fact that receptors with affinity for IgA and/or IgG are very common among clinical isolates of group A streptococci makes it particularly important to study their potential role in streptococcal disease, both with regard to primary infection and with regard to post-infectious sequelae. It is well known that group A streptococcal strains of different M type vary in nephritogenic and possibly also in rheumatogenic potential [18], and it seems possible that the type of immunoglobulin receptor expressed by a strain also influences the outcome of a streptococcal infection. The expression of these receptors by group A streptococci might also influence the outcome of immunoglobulin treatment, which has been considered as a possible therapy in serious infections [2].

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#### REFERENCES

1. Daly JD. Resurgence of group A streptococcal disease: Rheumatic fever. *Clin Microbiol Newslett* 1989; **11**: 161–3.
2. Stevens DL, Tanner MH, Winship J, Swartz R, Ries KM, Schlievert PM, Kaplan E. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N. Engl J Med* 1989; **321**: 2–7.
3. Gaworzewska E, Colman G. Changes in the patterns of infection caused by *Streptococcus pyogenes*. *Epidemiol Infect* 1988; **100**: 257–69.
4. Fischetti VA. Streptococcal M protein: Molecular design and biological behavior. *Clin Microbiol Rev* 1989; **2**: 285–314.
5. 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.
6. Christensen P, Oxelius VA. A reaction between some streptococci and IgA myeloma proteins. *Acta Path Microbiol Scand Sect C* 1975; **83**: 184–8.
7. Boyle MDP, Reis KJ. Bacterial Fc receptors. *Biotechnology* 1987; **5**: 697–703.
8. Lindahl G, Åkerström B, Frithz E, Hedén LO, Stenberg L. Protein Arp, the IgA receptor of group A streptococci. In: Boyle MDP, ed. *Bacterial immunoglobulin-binding proteins*. Vol I. New York: Academic Press, 1989: 193–200.
9. Svensson M, Christensen P, Schalén C. Monoclonal opsonic mouse antibodies specific for streptococcal IgG Fc-receptor. *J. Med Microbiol* 1986; **22**: 251–6.
10. Frithz E, Hedén LO, Lindahl G. Extensive sequence homology between IgA receptor and M proteins in *Streptococcus pyogenes*. *Mol Microbiol* 1989; **3**: 1111–9.
11. Lebrun L, Pillot J, Grangeot-Keros L, Rannou M-T. Detection of human Fc ( $\gamma$ ) receptors on streptococci by indirect immunofluorescence staining: a survey of streptococci freshly isolated from patients. *J Clin Microbiol* 1982; **16**: 200–1.
12. Schmidt KH, Kühnemund O, Köhler W. A screening of streptococci freshly isolated from human and animal sources for binding of human IgG. *Zbl Bakt Hyg A* 1987; **265**: 420–9.
13. Lindahl G, Åkerström B. Receptor for IgA in group A streptococci: cloning of the gene and characterization of the protein expressed in *Escherichia coli*. *Mol Microbiol* 1989; **3**: 239–47.
14. Martin PR, Høiby EA. Streptococcal serogroup A epidemic in Norway 1987–1988. *Scand J Inf Dis* 1990. In press.

15. Lindahl G. Cell surface proteins of a group A streptococcus type M4: The IgA receptor and a receptor related to M proteins are coded for by closely linked genes. *Mol Gen Genet* 1989; **216**: 372–9.
16. Health DG, Cleary PP. Cloning and expression of the gene for an immunoglobulin G Fc receptor protein from a group A streptococcus. *Infect Immun* 1987; **55**: 1233–8.
17. Åkesson P, Cooney J, Kishimoto F, Björck L. Protein H – a novel IgG binding bacterial protein. *Molec Immunol*. (In press).
18. Bisno AL. The concept of rheumatogenic and nonrheumatogenic group A streptococci. In: Read SE, Zabriskie JB, eds. *Streptococcal disease and the immune response*. New York: Academic Press, 1980; 789–803.