

Heterogeneity of Nonimmune Immunoglobulin Fc Reactivity Among Gram-Positive Cocci: Description of Three Major Types of Receptors for Human Immunoglobulin G

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Received for publication 18 April 1977

Two hundred and thirty strains of various gram-positive cocci were tested for quantitative, nonimmune binding of radiolabeled human polyclonal immunoglobulin G (IgG). The majority of coagulase-positive staphylococci and streptococci belonging to serogroups C and G showed a high uptake of IgG. The binding of immunoglobulin to group A streptococci was considerably less, with a number of strains completely negative. None of the pneumococcal or the group B or D streptococcal strains displayed any binding capacity. Heterogeneity of the IgG reactivity of various reactive strains was studied in an inhibition assay using 10 different animal serum pools. Three different inhibition patterns were seen, each of them revealing a striking degree of homogeneity within single bacterial species. *Staphylococcus aureus* and group A streptococci, respectively, constituted two homogeneous groups which differed markedly from each other and from C and G streptococci. No differences were observed between group C and G streptococci. Based on the profound differences between these homogeneous groups, three major types of Fc receptors could be defined. Type I and II Fc receptors were found on *S. aureus* and on group A streptococci, respectively. Fc receptor type III represented the immunoglobulin-binding structure of both group C and G streptococci.

Most strains of *Staphylococcus aureus* carry a surface component, protein A, with the capacity to combine with the Fc part of immunoglobulin molecules (12, 21, 24, 25). In humans, immunoglobulin G (IgG) subclasses 1, 2, and 4 are reactive (24). Strong evidence suggests that subclasses of human IgA and IgM are reactive as well (15). Furthermore, immunoglobulins from various mammalian species can also interact with protein A (23).

The immunoglobulin Fc-binding capacity of protein A has already been utilized in various fields of immunological technology. These methods include the coagglutination method for serological identification of microorganisms (4, 9, 19, 31), the use of stabilized staphylococci as a separation reagent in radioimmunoassays (10, 17) as antibody adsorbent (7), and labeled protein A as a marker for cell-bound immunoglobulin (1, 2, 7, 8, 13). The immunoglobulin reactivity of protein A might also play a role in host-parasite relationships (11).

Group A, C, and G streptococci carry an Fc-binding structure analogous to protein A (20). Sensitized sheep cell agglutinating properties have been reported to occur in group B and D streptococci (5) and in pneumococci (29). Pre-

vious studies have indicated differences in the immunoglobulin reactivity of staphylococci and group A, C, and G streptococci (20). The purpose of the present investigation was to study this suggested heterogeneity of Fc reactivities in a larger number of bacterial isolates. The possible existence of immunoglobulin-binding surface components with different specificities might expand the potentials of the use of such Fc reactivities in immunological techniques. Further clarification regarding immunoglobulin specificity and homogeneity within different bacterial species might also provide the basis for studies to determine the biological significance of nonimmune immunoglobulin reactivity.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. A total of 230 unselected strains were included in the present study: 30 strains of *S. aureus*, 40 strains of *Streptococcus pneumoniae*, and 30 group A, 40 group B, 30 group C, 30 group D, and 30 group G streptococci. All strains were isolated from routine specimens of human origin. Lancefield grouping of the streptococci was performed using the coagglutination method (4). A protein A-negative staphylococcal strain, Wood 46, served as a nonreactive control strain. Staphylococci were grown in tryptone broth,

and pneumococci and streptococci were cultured in Todd-Hewitt broth. After 16 h of incubation at 37°C, the bacteria were harvested and washed twice in phosphate-buffered saline, pH 7.2, containing 0.02% sodium azide. The optical density at 540 nm was measured, and the bacterial concentration was calculated from a standard curve and adjusted to 10⁹ organisms per ml.

Radiolabeling of human IgG preparation. Freeze-dried, pooled human immunoglobulin was purchased from AB Kabi (batch no. 33574). Labeling with ¹²⁵I was performed using the chloramine-T method (27). Five milligrams of IgG (1 mg/ml in phosphate-buffered saline, pH 7.4) was mixed with 0.5 mCi of carrier-free ¹²⁵I in a beaker cooled in an ice bath. After addition of 50 μ l of chloramine-T (5 mg/ml), the reaction was allowed to proceed for 8 min under stirring. The labeling process was stopped by addition of 100 μ l of Na₂S₂O₅ (5 mg/ml). Free, unreacted isotope was removed by gel filtration on a Sephadex G-25 column. After determination of the protein concentration with a modified Folin method (26), human serum albumin (0.05% final concentration) was added to prevent nonspecific adherence of IgG to the test tube wall.

Radioimmunological assays. (i) Quantitation of IgG binding. All bacterial strains were tested for maximum uptake of radiolabeled IgG. These tests were carried out in plastic tubes (70 by 11 mm; A/S Nunc, Roskilde, Denmark) by adding 2 \times 10⁸ bacteria (200- μ l suspension containing 10⁹ test organisms per ml) to 0.5 μ g of labeled IgG. After 1 h at room temperature, 2 ml of phosphate-buffered saline containing 0.02% sodium azide and 0.05% Tween 20 was added to each tube and the bacteria were spun down. The radioactivity in the pellet was measured in a Selectronic gamma spectrometer, and the uptake is expressed as percentage of total radioactivity added. An uptake of less than 10% was considered negative.

(ii) Inhibition assay. Highly reactive strains of staphylococci and streptococci belonging to serogroups A, C, and G (15 strains of each) were selected for use in an inhibition assay. In a pilot study, the inhibiting capacity of pooled normal human serum was determined. Increasing amounts of pooled normal human serum were mixed with 2 \times 10⁸ organisms, and 0.5 μ g of labeled IgG was then added. In an extended study, the inhibiting capacity of pools of serum samples from the following species was determined: human, rabbit, rat, guinea pig, dog, cat, horse, pig, sheep, cow, and chicken. Based on the results from the pilot study, the streptococci and the staphylococci were then tested with 4- and 8- μ l serum samples, respectively. These volumes represented the smallest amounts of human serum capable of giving complete inhibition in the assay. The test series using animal serum pools always included a determination of the maximum binding capacity of each strain. All test samples were processed as described for the uptake study. Nonspecific background adsorption was recorded in every experiment by testing the nonreactive strain Wood 46 simultaneously. Background counts were deducted from recorded uptake of IgG by the test strain. Inhibiting capacity of a serum sample was expressed as the difference between the maximum uptake and the uptake obtained in the inhibition assay.

To compare strains of different reactivity, it was necessary to express the degree of inhibition as percentage of maximum uptake of IgG. Less than 50% inhibition was considered negative.

RESULTS

Quantitative uptake of human IgG. Two hundred and thirty strains of gram-positive cocci of human origin were tested for binding of radiolabeled human IgG (Fig. 1). All 30 strains of *S. aureus* were highly reactive, with a capacity to bind 60 to 75% of the radioactivity in the IgG preparation. The 25 to 40% nonreactive radioactivity represents protein A-negative IgG-3 molecules, denatured IgG, and free iodine. There was rather little variation between different isolates, indicating a relatively homogeneous population with high affinity for IgG. None of the 40 pneumococcal isolates showed any reactivity. Additional tests on five pneumococcal strains using a larger excess of bacteria were also negative. Furthermore, 40 strains of group B streptococci and 30 strains of group D streptococci failed to demonstrate any binding of IgG. Among group A streptococci, 19 of 30 isolates were positive, with a varying degree of binding. The proportion of bound IgG did not exceed 40%. Twenty-five of 30 group C streptococci were capable of binding IgG. A high degree of reactivity was noted except for two strains. Group G streptococci showed a tendency toward a split population pattern. Twenty-five of 30 strains were strongly positive, with a binding capacity of 50 to 80% of added IgG, whereas the remaining five strains were negative.

Inhibition of IgG uptake by normal human serum. Human serum is capable of inhibiting the uptake of radiolabeled IgG to Fc-reactive strains. In a pilot study, the inhibiting capacity of pooled normal human serum was almost identical for strains of *S. aureus* and group A, C, and G streptococci (Fig. 2). Due to an apparent lower degree of reactivity, the group A curve was less steep. The uptake of IgG by all three groups of streptococci was reduced to one-half with serum samples of about 0.2 μ l. Complete inhibition was obtained using 5 μ l of serum. In contrast, staphylococcal strains required two to three times as much serum to achieve a comparable degree of inhibition. As the pilot study disclosed similarities between group C and G streptococci, three more strains of each of these groups were tested in a simplified inhibition assay using three serum samples. These inhibition curves were completely parallel.

Inhibition patterns using animal serum pools. To define possible differences in IgG-reactive structures, highly reactive strains were

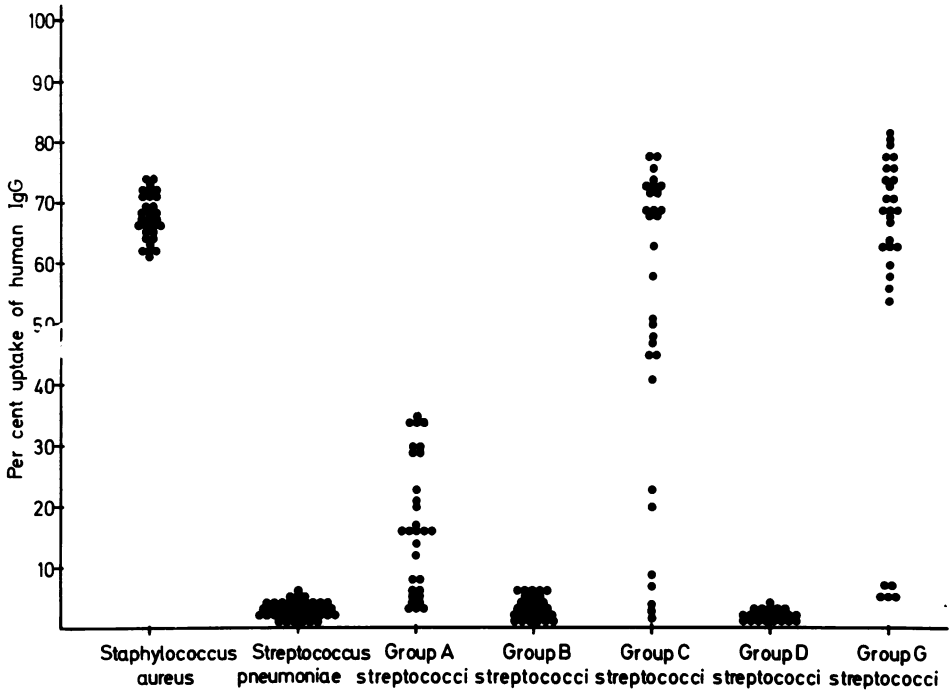


FIG. 1. Quantitative binding of human polyclonal IgG (0.5 μ g) to strains (2×10^8 organisms) of *S. aureus*, *S. pneumoniae*, and streptococci belonging to serogroups A, B, C, D, and G. The binding capacity is expressed as percentage of added radiolabeled IgG.

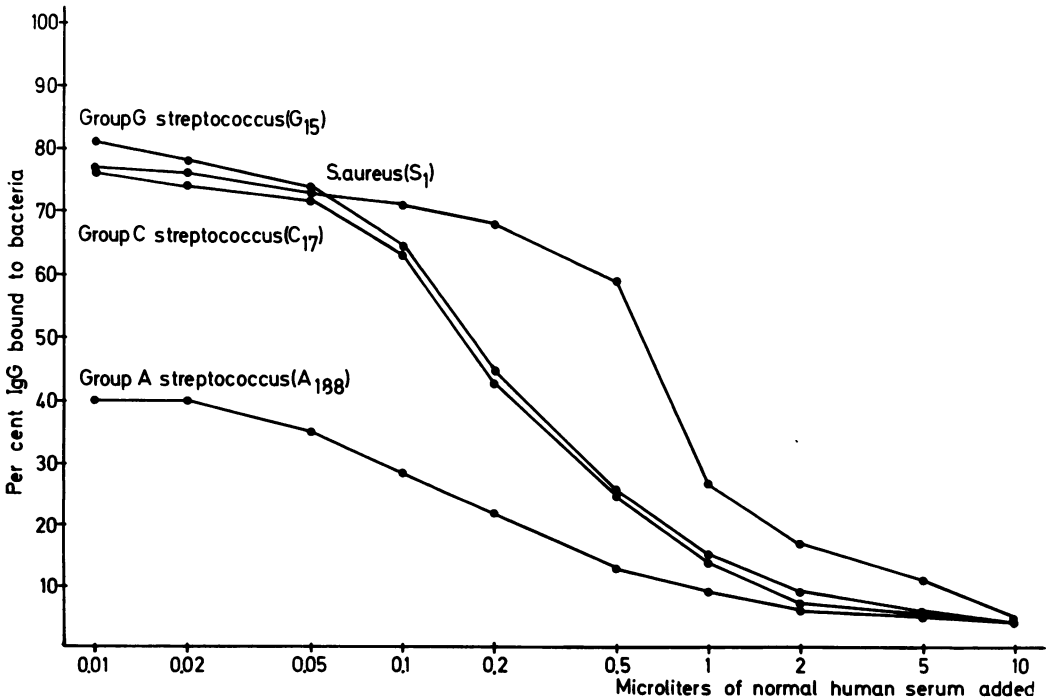


FIG. 2. Capacity of pooled human serum to inhibit the binding of radiolabeled IgG (0.5 μ g) to representative strains (2×10^8 organisms) of *S. aureus* and of group A, C, and G streptococci. The uptake is expressed as percentage of radioactivity bound to the bacterial pellet.

tested in a special inhibition assay. In this assay, serum pools from 10 different animal species were allowed to compete with radiolabeled IgG for binding to the strains tested. Streptococcal and staphylococcal strains were analyzed using 4- and 8- μ l serum samples, respectively. To allow for comparisons of strains of varying degrees of IgG reactivity, the observed inhibition was expressed as percentage of maximum achievable inhibition. Fifteen strains of *S. aureus* were quite homogeneous, with a high degree of inhibition, when tested with human, rabbit, guinea pig, dog, cat, and pig sera (Fig. 3A). Rat, horse, sheep, cow, and chicken sera were negative.

Studies of 15 strains of group A streptococci revealed inhibition of IgG uptake by serum samples from humans, rabbits, and pigs. All other serum pools were negative in the inhibition assay (Fig. 3B). Group C and G streptococci (15 strains of each group) behaved as one uniform population regarding the inhibition patterns (Fig. 3C and D). Serum pools from humans, rabbits, guinea pigs, horses, pigs, sheep, and cows gave strong inhibition. Rat, dog, cat, and chicken serum pools were negative. The results of the inhibition studies using animal sera are summarized in Table 1.

DISCUSSION

The immunoglobulin specificity of protein A, the Fc-binding structure of the staphylococcal cell surface, is rather well characterized as far as the Cowan I strain (NCTC 8530) is concerned (12, 15, 22-24). Data on other strains of *S. aureus*, however, are not available, and relatively little is known concerning the streptococcal Fc receptor (3, 6, 20). The present study was designed to elucidate differences of Fc receptors of various bacterial species. Our approach involved the use of a battery of animal serum pools in a standardized inhibition assay. As immunoglobulins from related species differ slightly, they might be used to identify minor variations in the structures of immunoglobulin receptors. Highly reactive strains of *S. aureus* and of group A, C, and G streptococci were tested in this assay, and the inhibition profiles were plotted (Fig. 3A-D). These plots showed that the strains belonging to the same bacterial species displayed surprisingly little variation. Each bacterial species tested seemed to constitute a homogeneous group of organisms with identical types of Fc receptors. When the four different species were compared, distinct variations were observed. On the basis of these characteristic differences, three types of Fc receptors for human IgG were defined (Table 1). This

differentiation refers to the inhibition profiles obtained using serum pools from guinea pigs, dogs, cats, horses, sheep, and cows. Type I and II immunoglobulin Fc receptors are found on *S. aureus* and on group A streptococci, respectively. The type III Fc receptor is the immunoglobulin-binding structure of both group C and G streptococci. Further inhibition tests of six group C and G streptococci strongly supported the close similarity of the Fc-reactive structures among these strains. The present studies of IgG-binding structures of the bacterial cell surface in gram-positive cocci were performed using a well-established assay technique (6, 20, 22). The sensitivity of the test procedure depends on the ratio between quantity of IgG and the number of bacterial organisms (22). All tests were carried out with an excess of bacteria. Under these circumstances almost all of the reactive IgG molecules will bind to the bacterial surface when the binding affinity is high. The reactive fraction of the radiolabeled IgG preparation can thus be determined. A small quantity will be trapped nonspecifically in the bacterial pellet. This fraction can be estimated by testing a nonreactive strain (Wood 46). In our experiments, 5 to 8% of the labeled immunoglobulin preparation was bound nonspecifically. Based on this observation, strains were considered as reactive when they were able to bind more than 10% of added IgG.

In the present studies group B and D streptococci failed to reveal any IgG-binding properties. This is in contradiction to other studies (5). Nonimmune Fc reactivity has been reported to occur also in pneumococci (29). The report was based on studies performed with an agglutination procedure using sensitized sheep erythrocytes (32). In the present investigation we were unable to detect IgG-binding properties in any of 40 different isolates. As the isotope method used in our experiment is more reliable than the agglutination procedure, B and D streptococci as well as pneumococcal strains can be considered completely negative.

Several microbiological characteristics are common to group A, C, and G streptococci isolated from infections in humans. However, there are some factors indicating a position for group A strains separate from C and G streptococci. M-proteins are limited to A strains, with rare exceptions (14, 28). Group A streptococci are found only in humans, whereas group C and G strains are common causative agents in animal infections. Acute glomerulonephritis and rheumatic fever occur as postinfectious complications only after group A streptococcal infections (30). Our studies have added another feature

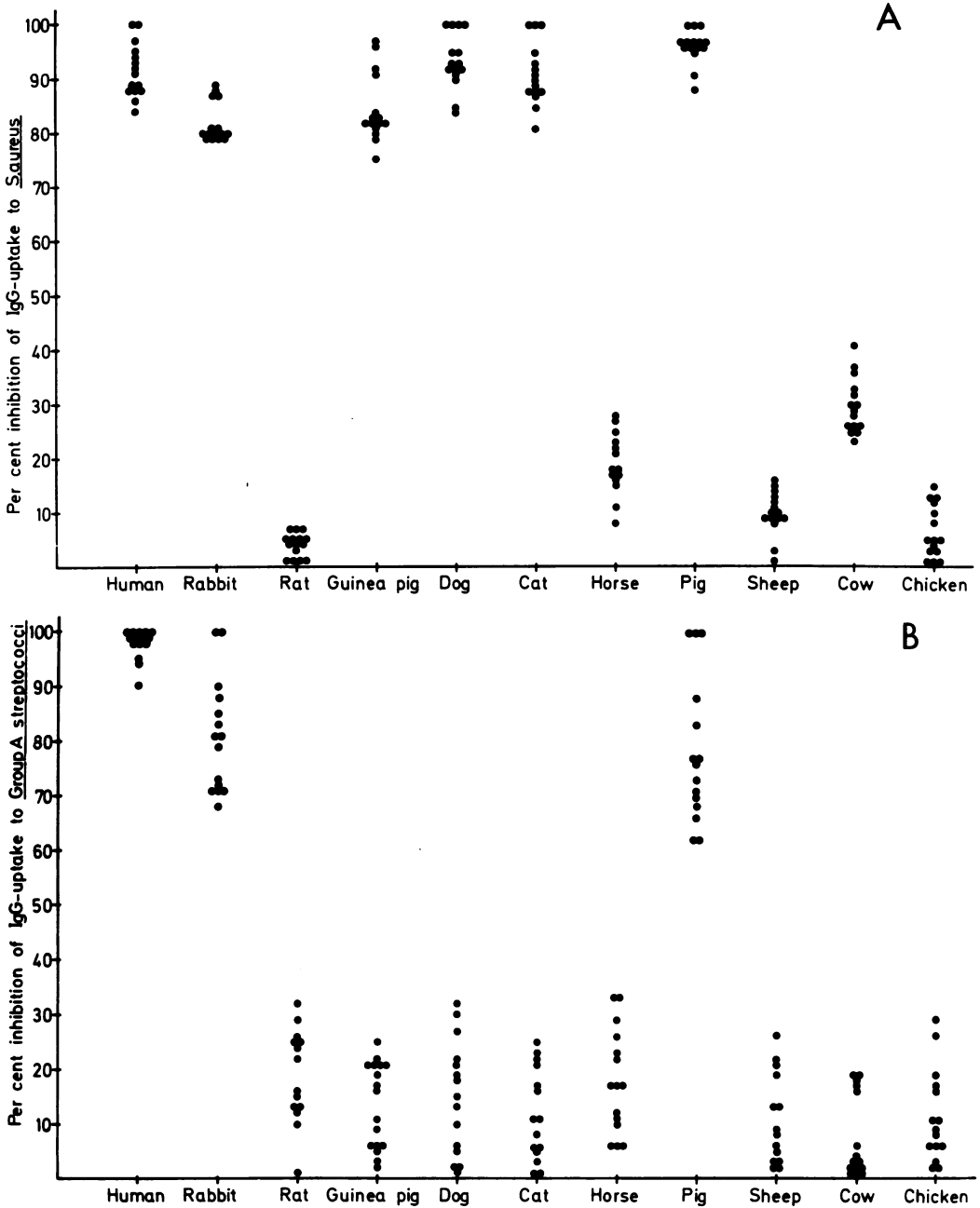


FIG. 3. Capacity of serum pools from various animal species to inhibit the uptake of radiolabeled IgG (0.5 μ g) to highly reactive strains (2×10^8 organisms). Inhibiting capacity is expressed as percentage of maximum obtainable inhibition. (A) Inhibition profile of 15 strains of *S. aureus* tested with 8- μ l serum samples. (B, C, and D) Inhibition profiles of 15 strains each of group A, C, and G streptococci tested with 4- μ l serum samples.

separating A strains from the other two groups. Both C and G streptococci carry a similar type of immunoglobulin Fc receptor, called type III, being markedly different from the type II Fc

receptor found on group A streptococci.

Protein A-carrying staphylococci are used for the separation of immunoglobulin-bound antigen from free antigen in radioimmunoassays

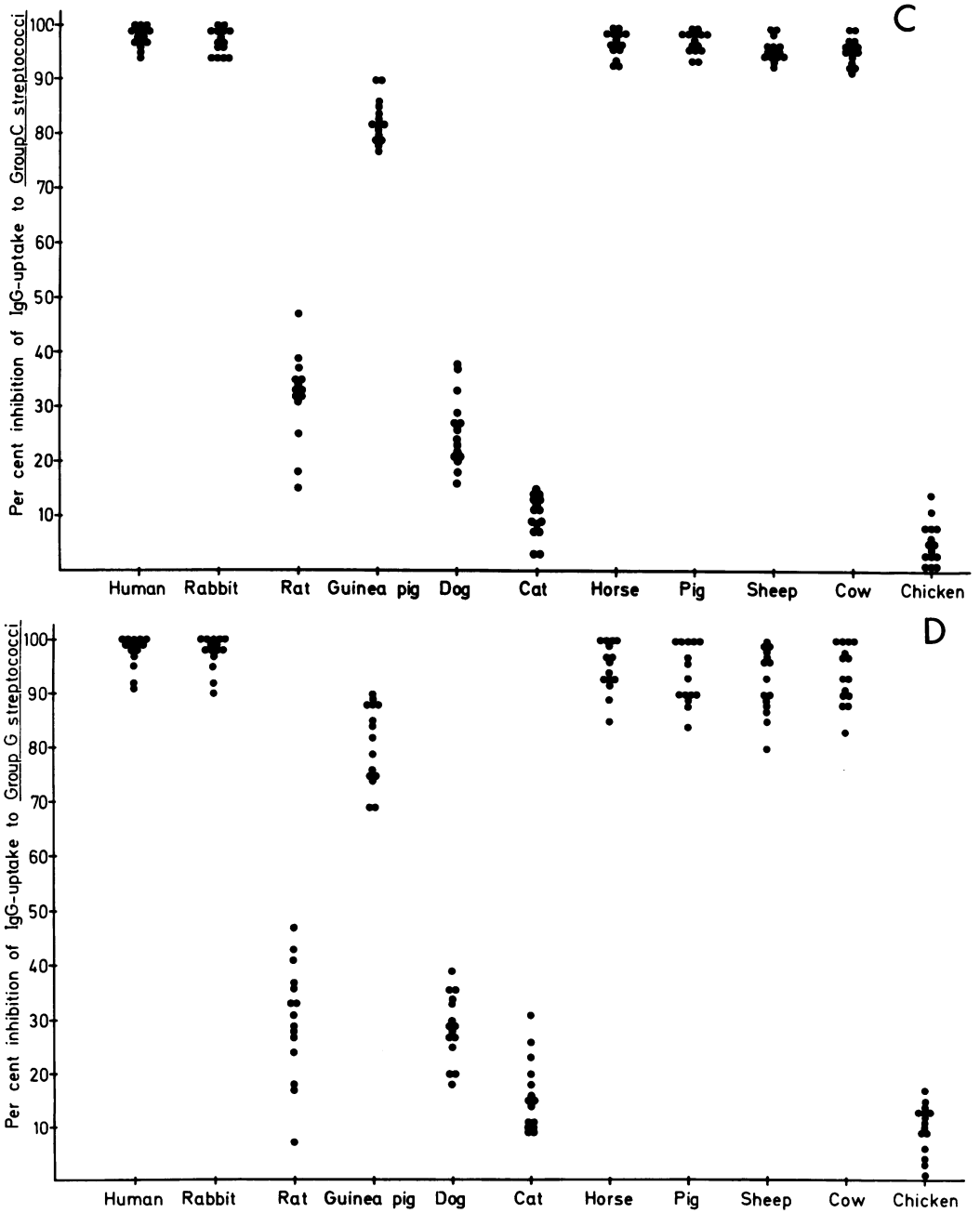


FIG. 3C and D.

(10, 17). Staphylococci coated with specific antibodies constitute coagglutination reagent for the identification of microorganisms and their soluble surface antigens (4, 9, 19, 31). Purified protein A can be labeled with fluorescein isothiocyanate, iodine, or ferritin and used for the detection of reactive immunoglobulin molecules

(1, 2, 7, 8, 13). Coupled to Sepharose 4B, protein A has been used for the isolation of IgG, particularly subclass IgG-3 (16). The use of protein A as exemplified is certainly far from fully exploited. The identification of similar but not identical Fc-binding structures could expand the present field of utilization. The demonstra-

TABLE 1. *Inhibiting capacity of various animal serum pools as studied in a standardized inhibition assay with 15 strains of each bacterial species^a*

Source of serum	<i>S. aureus</i>	Groups A streptococci	Group C streptococci	Group G streptococci
Humans	++	++	++	++
Rabbits	++	++	++	++
Rats	-	-	-	-
Guinea pigs ^b	++	-	++	-
Dogs ^b	++	-	-	-
Cats ^b	++	-	-	-
Horses ^b	-	-	++	++
Pigs	++	++	++	++
Sheep ^b	-	-	++	++
Cows ^b	-	-	++	++
Chickens	-	-	-	-
IgG Fc receptor type	I	II	III	III

^a ++, Inhibition exceeding 50%; -, inhibition less than 50%.

^b Differentiating serum sample.

tion of three different types of Fc receptors in the present study may therefore represent a significant step towards the definition of such similar Fc receptors. These receptors might, for instance, differ in their immunoglobulin class and subclass specificity, an aspect which would be of great interest to scientists concerned with the separation and characterization of immunoglobulins.

Attempts have been made to elucidate the biological significance of the IgG-binding property of protein A (11). Similar studies on Fc-reactive streptococci have not been reported. It is, however, quite clear that the Fc reactivity must be taken into account when aspects of acute streptococcal infections as well as the pathogenesis of postinfectious complications are studied.

ACKNOWLEDGMENTS

We extend our sincere thanks to Åke Christensson for making the illustrations and to Ingrid Lindhe-Johansson for typing the manuscript.

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