Specific Absorption of Human Serum Albumin, Immunoglobulin A, and Immunoglobulin G with Selected Strains of Group A and G Streptococci

GÖRAN KRONVALL,* ANTHONY SIMMONS,† ERLING B. MYHRE, AND SVANTE JONSSON

Department of Medical Microbiology, University of Lund, S-223 62 Lund, Sweden

Five gram-positive bacterial strains were selected for absorption studies of human serum samples. Strain AR1 (group A, M-type 1) and G148 (group G), with strong immunoglobulin G (IgG) binding capacities, and strain AW43 (group A, M-type 60), binding both IgA1 and IgA2, were compared with *Staphylococcus aureus* Cowan I and with *Staphylococcus epidermidis* L603. Both AR1 and G148 were capable of completely absorbing out serum IgG. In contrast, *S. aureus* Cowan I left a fraction unabsorbed, as expected from its known lack of IgG3 binding. Strain AW43 absorbed out all serum IgA, using a 10-µl bacterial pellet for 20 µl of serum. Serum IgM levels were slightly reduced by *S. aureus* Cowan I absorption. On the basis of the experiments, a bacterial mixture was designed consisting of *S. aureus* Cowan I and group A streptococcus strains AR1 and AW43, with absorption characteristics suitable for use in discriminating between early IgM and late IgG and IgA immune responses in routine serological work. A new type of bacteria-mammalian protein binding was discovered. Human serum albumin was completely absorbed out by strain G148 and to a lesser extent by strain AR1 and AW43. *S. aureus* Cowan I and *S. epidermidis* were negative. The binding capacity of G148 for albumin equalled that of Cowan I for IgG. The binding pattern of albumin to the strains was different from those of IgG, IgA, IgM, fibrinogen, haptoglobin, or aggregated β_2-microglobulin and therefore seems to represent another type of bacterial-mammalian interaction with a specific albumin receptor on the surface of streptococci.

Methods for separating long-standing immunoglobulin G (IgG) antibodies from IgM antibodies indicating a recent infection are of importance in many serological investigations. A new principle for such a discrimination suitable for routine use has been presented earlier (1). Heat- and formaldehyde-stabilized *Staphylococcus aureus* Cowan I, with a high content of protein A, was used for absorbing out IgG and leaving the majority of IgM and IgA in the samples. The method has been applied by others to rubella serology and to the diagnosis of herpes simplex infections (15, 37, 44, 45). Immunoglobulin-binding staphylococci have also been utilized in radioimmunoassays as originally described for the determination of α-fetoprotein (22, 23). This method has been applied to several other assays for antigens as well as for specific antibodies and also to the purification of cell surface proteins after solubilization and binding

† Present address: Department of Virology, The London Hospital Medical College, London E1 2AD, England.

to antibodies, a technique which offers certain advantages over other methods (4–6, 8, 10, 13, 18, 20, 21, 25).

In IgG-versus-IgM discrimination, as well as in more general immunoglobulin absorption techniques, there are certain limitations to the use of *S. aureus* Cowan I. Protein A of *S. aureus* will bind only IgG subclasses 1, 2, and 4, leaving specific IgG3 antibodies present unabsorbed in the serum samples (32). Recent studies have also indicated that a minor fraction of both IgA and IgM does in fact bind to staphylococcal protein A (14, 17, 35, 42, 43). In humans, the protein A-reactive fraction of IgA has been identified as the IgA2 subclass, and IgM2 has been proposed to designate the reactive fraction of IgM (17, 35, 42, 43). The fact that the major fraction of IgM is left unabsorbed explains the successful use of Cowan I staphylococci in serological work. Another limitation is the inability of staphylococci to bind antibodies belonging to the IgA1 subclass. A more complete immunoglobulin binding by bacteria, also IgG3 and IgA

antibodies, would permit a more widespread use of bacterial absorption procedures in serological work.

Alternative bacterial particles for immunoglobulin absorption are available since the discovery of Fc binding surface structures in group A, C, and G streptococci (27). In these first studies, a binding of IgG3 in addition to IgG1, -2, and -4 was noted, and the potential use of bacteria other than *S. aureus* in immunological work was pointed out. Human IgA is also taken up by many group A streptococci (7, 29). Both IgA subclasses A1 and A2 seem to bind to the reactive surface structures (E. Myhre, M. Harboe, and G. Kronvall, unpublished data) which are different from those binding IgG (7, 29). No significant IgA binding is seen with group C or G streptococci. IgM is not bound to any appreciable extent by streptococci, although a low-grade affinity can be detected in most strains (Myhre et al., unpublished data). The background information available indicated that selected streptococcal strains might serve as immunoglobulin-absorbing particles of potential use in laboratory work. The present studies have provided direct evidence for such use. In addition, a new type of bacterial-mammalian protein interaction was discovered. Human serum albumin was found to bind to streptococci, particularly to strain G148, with an absorbing capacity in the same range as that of *S. aureus* Cowan I for human IgG.

MATERIALS AND METHODS

Bacterial strains. Group A, C, and G streptococcal strains were initially screened for their capacity to bind several different human proteins as listed in Table 1 (26, 27, 29, 41; G. Kronvall, C. Schönbeck, and E. B. Myhre, submitted for publication). Strains with many different combinations of positive binding were found, and two group A strains (AR1, M-type 1; AW43,

TABLE 1. *Binding of radiolabeled human proteins to three streptococcal strains*[a]

Protein	AR1	AW43	G148
IgG	93	24	83
IgA1	9	47	7
IgA2	8	54	5
IgM1	14	12	10
IgM2	19	13	15
IgD	10	7	8
IgE	6	6	6
Fibrinogen	56	53	6
Haptoglobin	2	66	3
Aggregated β_2-microglobulin	79	17	3

[a] Binding is expressed as percentage of added radioactivity remaining in the bacterial pellet (27, 29, 31, 41; Kronvall et al., submitted for publication).

M-type 60) and one group G strain (G148; ref. 27) were selected for further studies. As seen in Table 1, the two group A strains were quite different regarding Ig specificity; AR1 bound IgG very strongly but little IgM and no IgA, whereas the AW43 strain showed strong binding of IgA1 and IgA2, less IgG, and little IgM. Strain G148 was quite similar to AR1 but was included because it represented a type III Fc receptor in contrast to the type II receptor in AR1 (41). Unpredictable differences, therefore, might show up. All three strains were negative regarding the IgD and IgE myeloma proteins tested. With respect to three other types of human protein binding, the strains represented three different combinations of these reactivities (26, 31; Kronvall et al., submitted for publication). *S. aureus* Cowan I (NCTC 8530) was included for comparisons with an established immunoglobulin-absorbing particle. *Staphylococcus epidermidis* L603, obtained from a specimen in our clinical microbiology laboratory, was used as a control strain in the studies because preliminary tests indicated a completely negative pattern against the human proteins tested.

Cultivation and stabilization of bacteria. Streptococcal strains were cultured in Todd-Hewitt broth using an apparatus for batch or continuous culture. A Teflon lid with eight connections was clamped onto a 5-liter glass vessel (Quickfit FC 51). A tight seal was obtained by using an O-ring (SOR-76). The culture vessel was heated by a surrounding coil of electrothermal cord (Electrothermal Engineering Ltd., London) connected to a transformer, and the temperature was controlled by an indicating temperature controller, YSI model 73 ATD (Yellow Springs Instrument Co., Yellow Springs, Ohio) with probe no. 402. The thermistor probe was put into a blind metal tube inserted through the lid. Automatic pH control was achieved using a Radiometer pH meter model no. 28, Titrator no. 11, and Auto-Burette ABU 16 with recorder (Radiometer, Copenhagen, Denmark). The combined electrode, GK 2302 C (Radiometer) was sterilized in ethanol containing 0.5% (vol/vol) concentrated H_2SO_4 and placed in a special O-ring-sealed connection in the Teflon lid. A magnetic stirrer equipped with an extra-strong magnet produced the necessary stirring of the medium.

A 1-liter volume of Todd-Hewitt broth was inoculated and incubated overnight at 37°C without stirring and used as an inoculum for the fermentor with 4 liters of fresh medium added. The pH was kept at 7.2 with automatic additions of 5 M NaOH. Sterile glucose (10%) was added in 10-ml portions during the cultivation to promote growth. Broth samples were taken at regular intervals, and the optical absorbance was read at 620 nm in a Beckman model CP-1 colorimeter to monitor bacterial growth. At the end of the log growth phase the continuous cultivation could be started at a rate of about 50 ml/min. Bacteria were harvested by centrifugation at 3,000 rpm for 20 min in a Beckman J-6 centrifuge with rotor JS-4.2, suspended in phosphate-buffered saline (0.02 M phosphate, pH 7.4, 0.13 M sodium chloride, 0.02% sodium azide) and killed by a heat treatment at 80°C for 5 min. The bacteria were then washed and suspended to 10% (vol/vol) in phosphate-buffered saline and stored at 4°C.

S. aureus Cowan I and *S. epidermidis* L603 were

cultured in CCY medium (2), 800 ml, in 2-liter whirl bottles for sufficient aeration. The bacteria were harvested, formalin- and heat-treated as described previously, and stored as a 10% suspension in phosphate-buffered saline at 4°C until use (28).

Absorption of serum samples. Samples of 10% bacterial suspensions, from 50 μl to 4 ml, in plastic tubes (70 by 12 mm; AB CERBO, Trollhättan, Sweden), were centrifuged at 2,000 × g in a Beckman J-6 centrifuge with a JR-3.2 rotor, giving bacterial pellets of defined volumes (5, 10, 20, 50, 100, 200, and 400 μl). Serum samples were diluted 10 times in 0.075 M Veronal buffer (pH 8.6) containing 0.002 M calcium lactate. Samples of diluted serum (200 μl unless otherwise stated) were added to bacterial pellets, and the bacteria were suspended using a Vortex-Genie mixer (Scientific Industries, Inc., Springfield, Mass.) and incubated at room temperature for 1 h. The suspensions were then centrifuged, and the supernatants were recovered for further analysis.

Quantitation of human albumin, IgG, IgA, and IgM. The concentrations of albumin, IgG, and IgA in the serum samples subjected to bacterial absorptions were determined using the rocket electrophoretic technique (33, 34). IgG and IgA were analyzed simultaneously using a mixed antiserum (34). Quantitation of IgM was performed using single radial immunodiffusion (38). A control serum sample with known concentrations of the proteins analyzed served as a reference. All antisera used were purchased from Dako A/S, Copenhagen, Denmark. Results were expressed as grams of protein per liter of the original, undiluted serum sample.

RESULTS

Bacterial absorption of serum IgG, IgA, and IgM. Diluted serum samples (200 μl, diluted 1:10) from an apparently healthy male adult, HC, were absorbed with increasing amounts of five different strains of gram-positive cocci: group A streptococcus AR1 and AW43, group G streptococcus G148 (see Table 1), *S. aureus* Cowan I, and *S. epidermidis* L603. The effect on concentrations of IgG, IgA, and IgM is shown in Fig. 1 to 3.

IgG was completely absorbed out by 100-μl pellets of strain AR1 and G148 (Fig. 1A and 2A and C). The other streptococcal strain included, AW43, showed a rather poor absorbing capacity with more than one tenth remaining even when using a 400-μl pellet. *S. epidermidis* L603 absorption values show a decreasing trend, which represents the dilution effect of the procedure at pellet volumes of 50 μl and above and hence serves as a background reference (Fig. 1A, Fig. 3B). The other absorption curves have not been corrected for this effect. The Cowan I curve (Fig. 1A) does not reach zero levels. This is expected from the known fact that IgG3 does not bind to protein A (32). The end point of about 0.8 g/liter is reached, however, using as little as a 20-μl

pellet of the bacteria (Fig. 3A). Highly reactive streptococci have to be used in larger quantities, 50 to 100 μl, to achieve the same result.

Immunoglobulin A quantitations revealed an exceptional performance of strain AW43 (Fig. 1B and 2B). As little as 10 μl of packed bacteria absorbed out all IgA present in the 20-μl serum sample diluted to a 200-μl volume. In contrast, the other four strains included in the studies left the IgA almost intact (Fig. 1B). In comparison with the L603 control, only a minor degree of absorption was noted. Therefore, strains AR1 and G148 are just as poor IgA-absorbing bacteria as Cowan I, in marked contrast to the AW43 strain. The essential differences of the absorbing capacities of the strains studied are demonstrated in Fig. 4.

IgM determinations of absorbed serum samples (200 μl of serum HC, diluted 1:10) showed interesting differences between *S. aureus* Cowan I and the streptococcal strains (Fig. 1C). Rather small bacterial pellets of Cowan I absorbed out part of the IgM, reaching a plateau with a 20-μl pellet of bacteria. The initial slope of the curve indicates relatively high affinity for this part of IgM, which in this case constitutes about half the total concentration. This selective absorption of some IgM removes the IgM2 subclass (14, 17, 35). Group A and G streptococci included in the studies showed no preferential binding of any IgM subclass (Table 1). The absorption curves (Fig. 1C) indicate a rather low avidity of streptococci for IgM, which is also reflected in low binding figures in direct measurements of uptake (Table 1). Even 400-μl pellets of strains AR1, AW43, and G148 were insufficient for a 50% absorption of the serum IgM present. The significant difference in the IgM levels after absorption with group A and G streptococci as compared to the control strain, L603, was only seen when using medium sized pellets, and not with larger bacterial volumes. It remains to be seen whether the kinetics of IgM binding are dependent on the relative saturation of receptors on the bacterial surface.

Serum samples (100-μl serum dilutions, 1:10) from five other healthy donors were also absorbed with streptococci to confirm the results obtained in the detailed analysis of serum HC. For comparative purposes, the results of immunoglobulin determinations (Fig. 5) were expressed as percent remaining in the supernatants. IgG concentrations were slightly lower after absorption with AR1 as compared to G148 (Fig. 5A). This slight but significant difference in IgG-absorbing capacity might explain the slightly higher binding figure in direct uptake measurements (Table 1), 93 and 83%, respec-
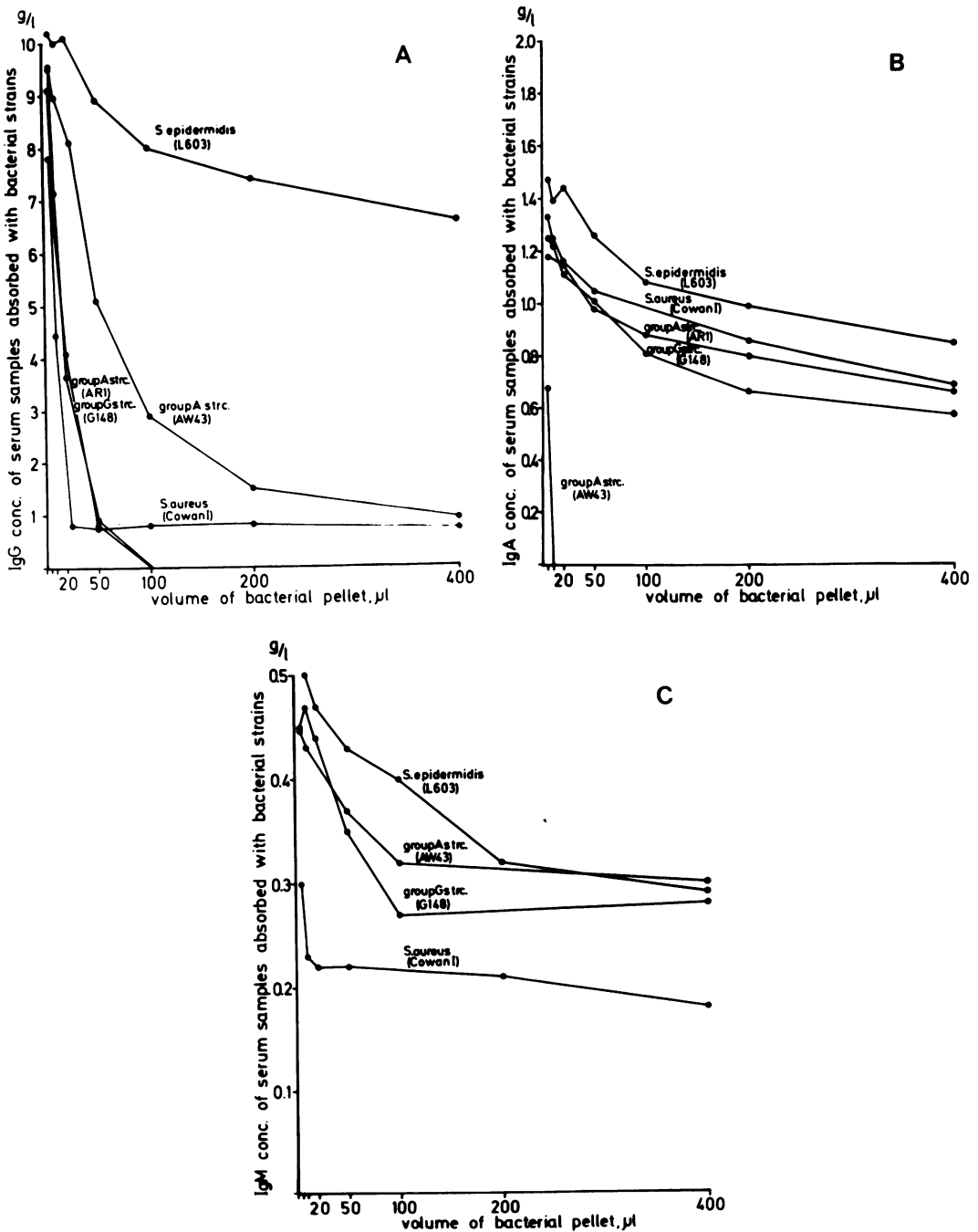
FIG. 1. *Quantitation of IgG (A), IgA (B), and IgM (C) in serum HC after absorption with varying volumes of bacterial pellets as indicated (5- to 400-μl pellets). Immunoglobulin levels are expressed in grams per liter of serum. In the absorption experiments 20-μl serum volumes were diluted to 200 μl and then added to the bacteria.*

tively. Regarding IgA, the strikingly high capacity of strain AW43 to bind both subclasses was confirmed in the absorption experiments (Fig. 5B). Strains AR1 and G148 were completely

ineffective for IgA absorptions. Immunoglobulin M levels of the five serum samples were essentially unchanged by absorptions with the streptococcal strains used, AR1, AW43, and G148
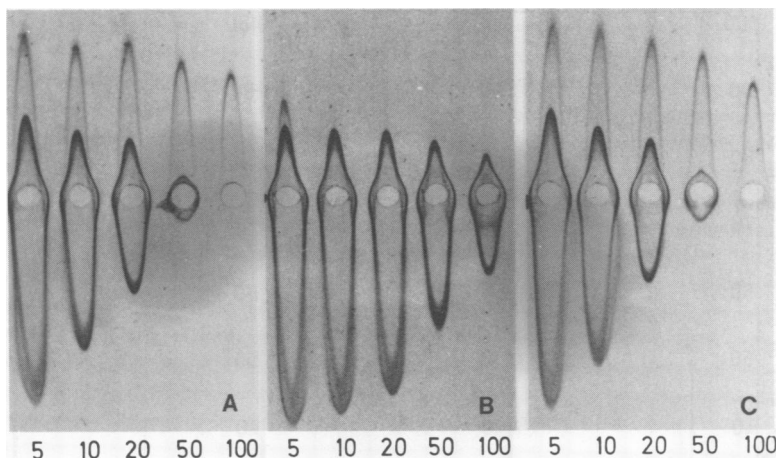
FIG. 2. *Examples of IgG and IgA quantitations in rocket electrophoresis of serum samples absorbed with streptococcus strain AR1 (A), strain AW43 (B), and strain G148 (C). Absorption with 100-μl pellets of AR1 and G148 gives a total depletion of IgG (A, C). From 20-μl volumes of AW43 no IgA is detectable (B).*
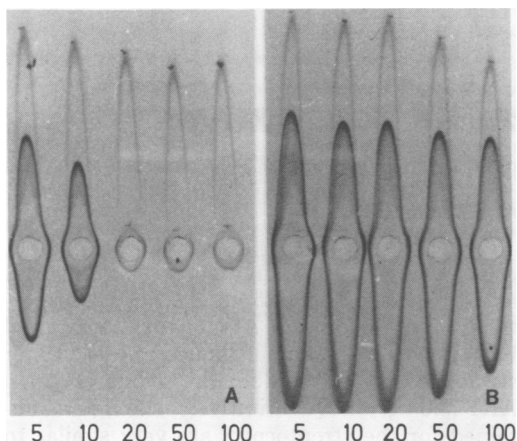

FIG. 3. *Examples of IgG and IgA quantitations after absorption of serum HC with S. aureus Cowan I (A) and S. epidermidis L603 (B). Note the presence of IgG in all Cowan I-absorbed samples. The slight reductions after L603 absorptions are due to the dilution effect of buffer in the large pellet volumes used.*
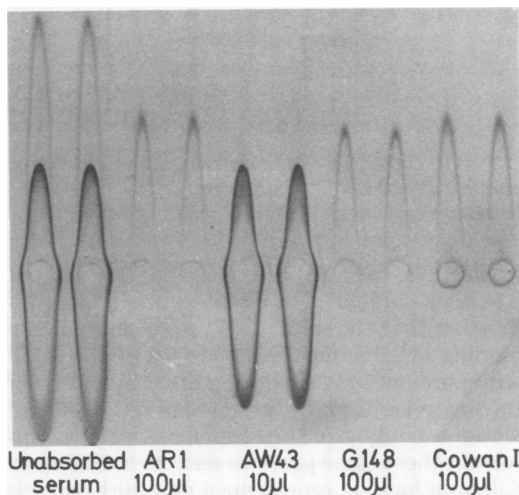
FIG. 4. *IgG and IgA levels in serum HC after absorption with gram-positive strains as indicated, showing complete depletion of IgG using strain AR1 and G148, the disappearance of IgA using AW43, and the remaining protein A-negative fraction using Cowan I.*

(Fig. 5C).

Bacterial absorption of human serum albumin. Quantitation of human serum albumin in serum samples absorbed with the five gram-positive cocci used throughout the studies was initially included for measuring the dilution of samples in the absorption procedures. The results, however, unexpectedly showed a definite binding of albumin to some of the strains (Fig. 6 to 8). Group G streptococcus strain G148 was extremely effective in absorbing out albumin, resulting in a complete depletion of the albumin content of the sample when using a 200-μl bac-

terial pellet (Fig. 6 and 7C). The two group A streptococci showed significant binding but to a lesser extent; strain AR1 removed somewhat more than strain AW43 (Fig. 7A and B, Fig. 8). The *S. epidermidis* curve reflected the dilution effect of the absorption procedure. *S. aureus* Cowan I was equally negative in these albumin absorption studies (Fig. 7D).

The possibility that the heating procedure for killing the bacteria used in the experiments might induce changes favoring a nonspecific absorption of albumin was also considered. How-
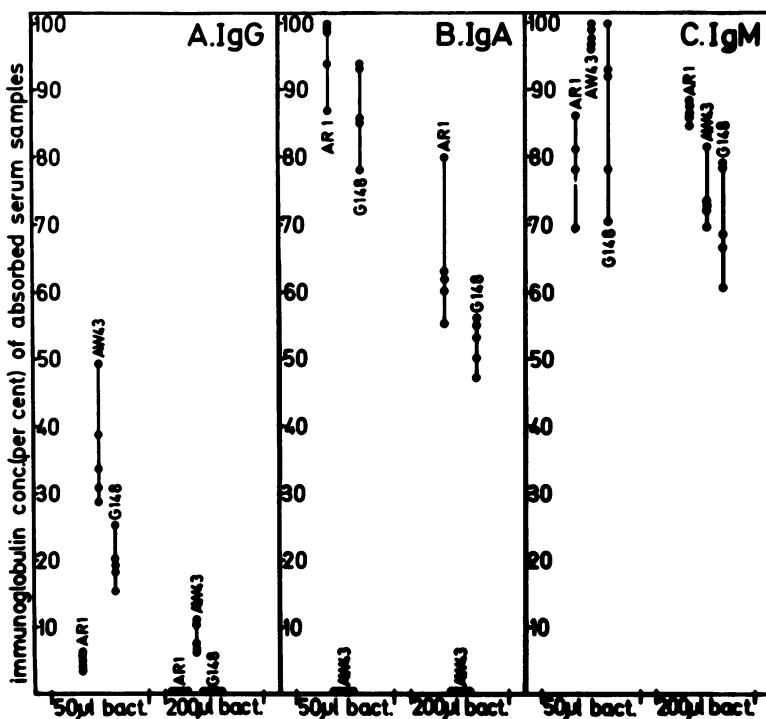
FIG. 5. *Immunoglobulin determinations in five serum samples (100 μl, diluted 1:10) absorbed with 50-μl and 200-μl pellets of streptococcal strains AR1, AW43, and G148, as indicated. IgG (A), IgA (B), and IgM (C) levels are expressed as percentages of a reference normal human serum sample.*

ever, a live, fresh culture of strain G148 showed a similar binding of human serum albumin, indicating that the structures responsible for the binding of albumin to the surface of the G148 strain are not induced during the preparation of the bacteria. From the patterns of IgG, IgA, and albumin binding to the strains used, the receptors for these three proteins seem to be different. Albumin binding represents a new, hitherto unrecognized type of mammalian-bacterial interaction.

Quantitative comparisons between absorbing capacities. The bacterial pellets required to reduce the concentrations of albumin by 25 g/liter, IgA by 0.7 g/liter, and IgG by 6 g/liter were determined from results according to Fig. 1. From these figures comparative absorbing capacities of the strains were calculated. Calculations using complete absorption as the end point are highly influenced by the different affinities of the interactions. The present figures serve as comparative values, which are more dependent on the maximal number of binding sites and to a lesser degree on the binding constants. The highest figures noted are for IgG absorption to Cowan I (10.7 μg/μl of pellet), and also for albumin binding to strain G148 (10.3 μg/

μl of pellet). IgG binding figures to strain AR1 and G148 rank second (5.7 and 6.3 μg/μl, respectively). IgA binding to AW43 with 2.9 μg/μl of bacterial pellet was unchallenged by the other strains, equaling the albumin binding of strain AR1 (3.0 μg/μl). Quantitatively, the figures obtained for the streptococci are very similar to those obtained for Cowan I absorption of IgG. These results further emphasize the potential role of streptococcal strains for immunoglobulin-binding procedures. The discovery of albumin binding might also have some practical applications.

DISCUSSION

The results of our studies have shown that streptococcal strains can serve as very efficient absorbing particles for the selective removal of IgG and IgA. Strains AR1 and G148 represent the most potent IgG-absorbing streptococci, with a capacity to bind all four IgG subclasses. Strain AW43 is capable of removing IgA with little IgG and negligible IgM binding. By the use of these strains the major limitations of staphylococcal protein A absorptions are eliminated. *S. aureus* Cowan I absorption is currently used for discriminating recent IgM response from long-
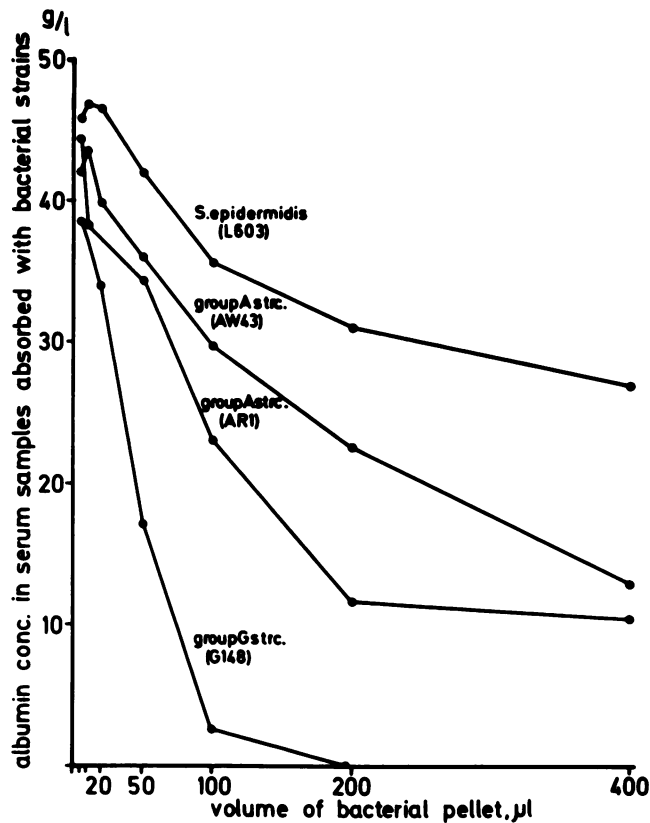
FIG. 6. *Quantitation of human serum albumin in serum HC after absorption with increasing pellet volumes of gram-positive strains as indicated.*
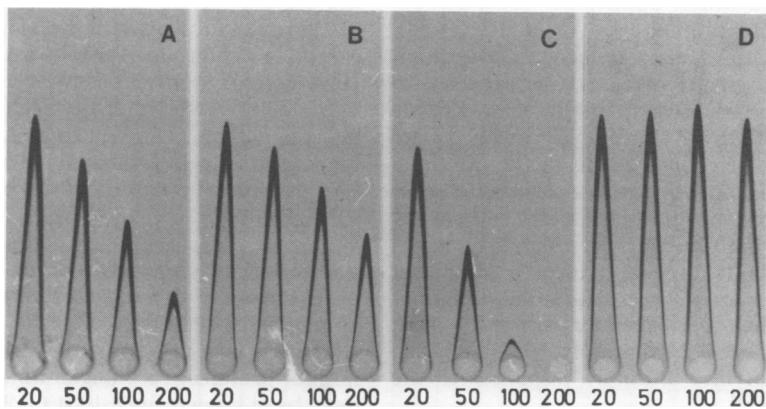

FIG. 7. *Examples of albumin quantitations in serum HC showing the complete depletion using strain G148 (C) as compared to the completely negative, protein A-rich strain Cowan I (D). (A) Streptococcus strain AR1; (B) streptococcus strain AW43.*

standing IgG antibodies (1, 15, 37, 45). These particles are not capable of binding IgG3 or IgA1 (17, 32, 42, 43), and the presence of late responses of these subclasses might therefore make IgM-versus-IgG and IgA discriminations difficult.

Also, the binding of IgM2 to staphylococci might cause problems (14, 17, 35). In terms of specificity, streptococci are therefore at an advantage. One aspect, however, speaks in favor of staphylococci: the smaller pellet required for maximal
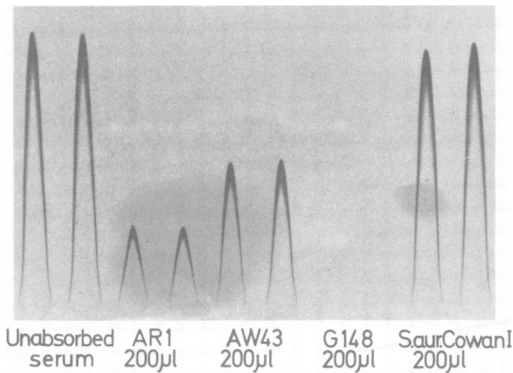
FIG. 8. *Demonstration of the albumin-absorbing capacity of three streptococcal strains, AR1, AW43, and G148, used for the absorption of 20 μl of serum HC diluted to 200 μl, as compared to unabsorbed serum and a Cowan I-absorbed control.*

absorption. For practical purposes a combination of 40 μl of AR1 bacteria, 20 μl of AW43, and 40 μl of Cowan I could be used together for the absorption of 200 μl of a 1:10 serum dilution. This mixture would meet the demand for a more generally applicable reagent for use in procedures to distinguish between early and late stages of the immune responses.

In radioimmunoassays, the method used for the separation of free antigen from antibody-bound antigen has to be chosen from several available ones, partly depending on the nature of the antigen. Absorption with *S. aureus* Cowan I constitutes a widely applicable separation reagent in radioimmunoassays (5, 6, 10, 20–23). Another advantage of staphylococcal absorption that was recognized early is the capacity to detect antibodies present in the same sample (23). Some other applications for antibody determinations have been made (5, 16, 30). The use of bacterial absorption in antibody determinations also requires particles with a sufficiently broad spectrum. In this regard, streptococci might be preferable to staphylococci. Also, a set of absorbing particles with different immunoglobulin-binding patterns, for example, AR1, AW43, and Cowan I separately, might provide further insight into antibody responses to the antigens studied.

The streptococcal strains used in the present studies are of two different IgG Fc-binding receptor types, as they have been defined earlier (41). Strain AR1 carries a type II receptor and G148 a type III. As far as human IgG and its subclasses are concerned, there is no major difference between type II and type III. However, when applied to animal species other than humans, the marked differences between type II

and III binding properties have to be considered. A fourth type of IgG Fc receptor has recently been defined in bovine β-hemolytic group G streptococci (40). It is possible that additional receptor specificities might expand the potential use of bacteria for selective immunoglobulin binding in various animal species.

Bacterial absorption procedures are routinely used in the production of M-type specific antisera. Nonimmune immunoglobulin-binding surface structures found on most group A streptococci might be expected to cause a decrease in IgG of the antisera on repeated absorptions and possibly also a fall in specific titers. Most group A strains show an IgG-binding capacity of the same degree as that shown by strain AW43 used in our studies. We can therefore estimate the influence on IgG levels of absorption procedures. One absorption with equal volumes of bacteria and undiluted serum would reduce the IgG concentration of human serum by about 15%. Three such procedures would still leave 60% of serum IgG unabsorbed. An even less conspicuous effect on rabbit IgG can be expected, since rabbit serum IgG shows a somewhat lower degree of binding to group A streptococci than human IgG (27, 41). However, occasional group A strains with stronger IgG-absorbing capacities, e.g. AR1 in the present studies, might cause nonimmune depletion of immunoglobulins in M-type specific antiserum production.

A new type of bacterial-mammalian protein interaction was discovered in the present studies. Human serum albumin was bound extensively to the group G strain G148 and to a lesser extent to the group A strains, AR1 and AW43 (Fig. 6 to 8). *S. aureus* Cowan I and *S. epidermidis* L603 were completely negative. The binding capacity of strain G148 was equal to the IgG binding of Cowan I, in terms of amount of protein per microliter of bacterial pellet. Albumin binding did not correlate with IgG or IgA binding and might therefore be considered to depend on a different streptococcal surface receptor. The exact nature of this receptor and its occurrence among bacterial strains are not yet known.

Streptococci are now known to bind several human proteins. Fibrinogen-binding properties have been known since 1934 (9, 24, 46; Kronvall et al., submitted for publication). IgG and IgA binding seems to depend on separate surface structures (7, 27, 29; Myhre et al., unpublished data). Aggregated β_2-microglobulin shows an affinity for group A, C, and G streptococci (29, 31). Haptoglobin can bind to some strains of streptococci (26). Albumin binding adds another type of interaction to this growing list. Also other bacteria possess different types of surface recep-

tors for human protein structures. Staphylococci can bind various immunoglobulins and fibrinogen (11, 19, 39). Complement component C1q will bind to gram-negative bacteria (36), as will aggregated IgG to lipopolysaccharide (12). Human leukocyte antigen-containing liposomes, HLA antigens, are bound to many bacterial species (25a). Several species are also mitogenic for human B lymphocytes (3). The presence of these quite specific animal-microbe interactions have to be considered in studies of parasite-host interactions. So far, they have been largely unmapped, and the number of microbe-animal interactions detected might increase considerably as this area becomes the subject of more extensive studies. As far as streptococci are concerned, their remarkable capacity to bind various serum proteins might provide a mechanism for evading recognition by the host through normal defense mechanisms.

LITERATURE CITED

1. Ankerst, J., P. Christensen, L. Kjellén, and G. Kronvall. 1974. A routine diagnostic test for IgA and IgM antibodies to rubella virus: adsorption of IgG with *Staphylococcus aureus*. J. Infect. Dis. 130:268–273.
2. Arvidson, S., T. Holme, and T. Wadström. 1971. Influence of cultivation conditions on the production of extracellular proteins by *Staphylococcus aureus*. Acta Pathol. Microbiol. Scand. Sect. B 79:399–405.
3. Banck, G., and A. Forsgren. 1978. Many bacterial species are mitogenic for human blood B lymphocytes. Scand. J. Immunol. 8:347–354.
4. Brown, J. P., J. M. Klitzman, and I. Hellström. 1978. Radioimmunoassay of murine leukemia virus p30 using *Staphylococcus aureus* is immunoadsorbent. J. Immunol. Methods 21:23–33.
5. Brunda, M. J., P. Minden, T. R. Sharpton, J. K. McClutchy, and R. S. Farr. 1977. Precipitation of radiolabelled antigen-antibody complexes with protein A-containing *Staphylococcus aureus*. J. Immunol. 119:193–198.
6. Brunner, H., W. Schaeg, U. Brück, U. Schummer, and H. G. Schiefer. 1977. A staphylococcal radioimmunoassay for detection of antibodies to *Mycoplasma pneumoniae*. Med. Microbiol. Immunol. 163:25–35.
7. Christensen, P., and V.-A. Oxelius. 1975. A reaction between some streptococci and IgA myeloma proteins. Acta Pathol. Microbiol. Scand. 83:184–188.
8. Cullen, S. E., and B. D. Schwartz. 1976. An improved method for isolation of H-2 and Ia alloantigens with immunoprecipitation induced by protein A-bearing staphylococci. J. Immunol. 117:136–142.
9. Duthie, E. S. 1955. The action of fibrinogen on certain pathogenic cocci. J. Gen. Microbiol. 13:383–393.
10. Figenschau, K. J., and J. C. Ulstrup. 1974. Staphylococcal radioimmunoassay for hepatitis B antigen and antibody. Acta Pathol. Microbiol. Scand. Sect. B 82:422–428.
11. Forsgren, A., and J. Sjöquist. 1966. Protein A from *S. aureus*. I. Pseudo-immune reaction with human γ-globulin. J. Immun. 97:822–827.
12. Ginsberg, M. H., and D. C. Morrison. 1978. The selective binding of aggregated IgG to lipid A-rich bacterial lipopolysaccharides. J. Immunol. 120:317–319.
13. Goding, J. W. 1978. Use of staphylococcal protein A as an immunological reagent. J. Immunol. Methods 20:241–253.
14. Grov, A. 1975. Human IgM interacting with staphylococcal protein A. Acta Pathol. Microbiol. Scand. Sect. C 83:173–176.
15. Handsker, R., and A. Fogel. 1977. Modified staphylococcal absorption method used for detecting rubella-specific immunoglobulin M antibodies during a rubella epidemic. J. Clin. Microbiol. 5:588–592.
16. Harboe, M., O. Closs, G. Bjune, G. Kronvall, and N. H. Axelsen. 1978. *Mycobacterium leprae* specific antibodies detected by radioimmunoassay. Scand. J. Immunol. 7:111–120.
17. Harboe, M., and I. Fölling. 1974. Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. Scand. J. Immunol. 3:471–482.
18. Jahrling, P. B., R. A. Hesse, and J. F. Metzgar. 1978. Radioimmunoassay for quantitation of antibodies to alphaviruses with staphylococcal protein A. J. Clin. Microbiol. 8:54–60.
19. Johansson, S. G. O., and M. Inganäs. 1978. Interaction of polyclonal human IgE with protein A from *Staphylococcus aureus*. Immunol. Rev. 41:248–260.
20. Jonsson, S. 1978. Protein-A-containing *Staphylococcus aureus* as an immunoglobulin-binding reagent in radioimmunoassay and in a non-radioactive surface immunoassay, p. 161–175. *In* Radioimmunoassay and related procedures in medicine, vol. 1. IAEA, Vienna.
21. Jonsson, S., G. Kahlmeter, T. Hallberg, and G. Kronvall. 1974. Characteristics of a new radioimmunoassay for gentamycin using ^{125}I-labeled gentamycin-HSA as a tracer and protein A-containing *Staphylococcus aureus* as a separation reagent, p. 160–163. *In* Sixth International Congress on Infection and Parasitic Diseases, reprints, vol. III, Warsaw.
22. Jonsson, S., and G. Kronvall. 1972. Protein A containing *Staphylococcus aureus* as anti-gammaglobulin reagent in radioimmunoassays. Scand. J. Immunol. 1:414–415.
23. Jonsson, S., and G. Kronvall. 1974. The use of protein A-containing *Staphylococcus aureus* as a solid phase anti-IgG reagent in radioimmunoassays as exemplified in the quantitation of α-fetoprotein in normal human adult serum. Eur. J. Immunol. 4:29–33.
24. Kantor, F. S. 1965. Fibrinogen precipitation by streptococcal M protein. I. Identity of the reactants, and stoichiometry of the reaction. J. Exp. Med. 121:849–859.
25. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. 115:1617–1624.
25a. Klareskog, L., G. Banck, A. Forsgren, and P. A. Peterson. 1978. Binding of HLA antigen-containing liposomes to bacteria. Proc. Natl. Acad. Sci. U.S.A. 75:6197–6201.
26. Köhler, W., and O. Prokop. 1978. Relationship between haptoglobin and *Streptococcus pyogenes* T4 antigens. Nature (London) 271:373.
27. Kronvall, G. 1973. A surface component in group A, C and G streptococci with non-immune reactivity for immunoglobulin G. J. Immunol. 111:1401–1406.
28. Kronvall, G. 1973. A rapid slide-agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. J. Med.

Microbiol. 6:187–190.

29. Kronvall, G., L. Björck, E. Myhre, and L. Wanna-maker. 1979. Binding of aggregated β_2-microglobulin, IgG, IgA and fibrinogen to group A, C and G strepto-cocci with special references to streptococcal M-protein. Proceedings of the VII International Symposium on Streptococci and Streptococcal Diseases.

30. Kronvall, G., G. Bjune, J. Stanford, S. Menzel, and D. Samuel. 1975. Mycobacterial antigens in antibody responses of leprosy patients. Int. J. Lepr. 43:299–306.

31. Kronvall, G., E. B. Myhre, L. Björck, and I. Berggård. 1978. Binding of aggregated human β_2-microglobulin to surface protein structure in groups A, C, and G strep-tococci. Infect. Immun. 22:136–142.

32. Kronvall, G., and R. C. Williams, Jr. 1969. Differences in anti-protein A activity among IgG subgroups. J. Immun. 103:828–833.

33. Laurell, C.-B. 1965. Antigen-antibody crossed electro-phoresis. Anal. Biochem. 10:358–361.

34. Laurell, C.-B. 1972. Electroimmuno assay. Scand. J. Clin. Lab. Invest. 29(Suppl. 124):21–37.

35. Lind, I., M. Harboe, and I. Fölling. 1975. Protein A reactivity of two distinct groups of human monoclonal IgM. Scand. J. Immunol. 4:843–848.

36. Loos, M., B. Wellek, R. Thesen, and W. Opperkuch. 1978. Antibody-independent interaction of the first component of complement with gram-negative bacteria. Infect. Immun. 22:5–9.

37. Mallinson, H., C. Roberts, and G. B. Bruce. 1976. Staphylococcal protein A; its preparation and an appli-cation to rubella serology. J. Clin. Pathol. 29:999–1002.

38. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by sin-gle radial immunodiffusion. Immunochemistry 2:235–254.

39. Much, H. 1908. Uber eine Vorstufe des Fibrinfermentes in kulturen von Staphylokokkus Aureus. Biochem. Z. 14:143–155.

40. Myhre, E. B., O. Holmberg, and G. Kronvall. 1979. Immunoglobulin-binding structure on bovine group G streptococci different from type III Fc receptors on human group G streptococci. Infect. Immun. 23:1–7.

41. Myhre, E. B., and G. Kronvall. 1977. Heterogeneity of non-immune immunoglobulin Fc reactivity among gram-positive cocci: description of three major types of receptors for human immunoglobulin G. Infect. Immun. 17:475–482.

42. Patrick, C. C., G. Virella, J. Koistinen, and H. H. Fudenberg. 1977. Differential binding of IgA proteins of different subclasses and allotypes to staphylococcal protein A. Z. Immun. Forsch. 153:466–469.

43. Saltvedt, E., and M. Harboe. 1976. Binding of IgA to protein A containing staphylococci: relationship to sub-classes. Scand. J. Immunol. 5:1103–1108.

44. Skaug, K., and P. I. Gaarder. 1978. An indirect immu-nofluorescent antibody test for determination of rubella virus specific IgM antibodies. Elimination of secondary IgM rheumatoid factor staining after absorption of se-rum IgG with staphylococcal protein A. Acta Pathol. Microbiol. Scand. Sect. C 86:33–35.

45. Skaug, K., and E. Tjotta. 1974. Diagnosis of recent herpes simplex infections. A modified immunofluores-cent test for the detection of specific herpes simplex IgM antibodies after staphylococcal adsorption of IgG. Acta Pathol. Microbiol. Scand. Sect. B 82:323–328.

46. Tillett, W. S., and R. L. Garner. 1934. The agglutination of hemolytic streptococci by plasma and fibrinogen. A comparison of the phenomenon to serological reactions with the same organisms. Bull. Johns Hopkins Hosp. 54:145–156.