INFECTION AND IMMUNITY, June 1990, p. 1789–1795
0019-9567/90/061789-07$02.00/0

Vol. 58, No. 6

Nonimmune Binding of Human Immunoglobulin A to Type II Group B Streptococci

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Received 14 August 1989/Accepted 14 March 1990

The binding of ^{125}I-labeled human myeloma immunoglobulin A (IgA) to four type II strains and one nontypable strain of group B streptococci was measured after streptococcal chains were broken by brief sonication. Some IgA binding was observed with all strains, but specific binding (binding that was inhibited by excess unlabeled IgA, was dose dependent, and was saturable) occurred only with those strains possessing the trypsin-sensitive beta component of the c protein. Similar amounts of binding were observed with myeloma IgA and IgA1 purified from normal serum. Specific binding was more rapid at 25°C than at 0 or 37°C and reached a plateau in 6 to 8 h. Binding was drastically reduced (85 to 90%) when streptococci had been heated (90°C for 1 h). Most radioactivity bound to group B streptococci could be displaced (>60% in 3 days) by the addition of excess unlabeled IgA. The binding capacity of one strain (10^8 streptococci in 1 ml of buffer) was saturated by approximately 24 μg of IgA. When transformed for Scatchard analysis, these data indicated that there was a specific binding capacity of 16,000 molecules of monomeric serum IgA per single streptococcal cell. The dissociation constant for IgA binding was 19.3 nM. Since enzyme-linked immunosorbent assay studies showed that the myeloma IgA used for the studies described above was IgA1, our quantitative data apply only to the binding of this subclass to group B streptococci. However, an enzyme-linked immunosorbent–filtration assay showed that both IgA1 and IgA2 bound to a type II group B streptococcus bearing the c protein.

The c protein (or Ibc protein) is a cell wall structure found in many group B streptococci (GBS) and is a useful marker for classification of GBS by precipitin typing (11). For example, both type Ia strains and type Ia/c (or Ic) strains possess the type Ia capsular polysaccharide and are differentiated by the presence of the c protein in the latter strains. The c protein is also found in type Ib GBS and about one-half of the type II strains, but is rarely found in type III GBS (12, 24). Wilkinson and Eagon showed that this protein is composed of two antigenically distinct components, one trypsin resistant (TR) and the other trypsin sensitive (TS) (25), corresponding to the alpha and beta antigens, respectively, described by Bevanger and Maeland (4). Brady et al. recently detected two additional c-protein determinants (gamma and delta) on the surfaces of some GBS by using a radioimmunoassay (6).

Russell-Jones et al. reported that some GBS with the c protein exhibit nonimmune binding of human immunoglobulin A (IgA), apparently because of the interaction of the Fc region of IgA with the TS (β) antigen of c protein (23). Binding of IgA to this component was also demonstrated in cloning experiments by Cleat and Timmis (7). In addition, Brady and Boyle demonstrated IgA binding to both the TS (β) component on the bacterial surface and the TS (β) component secreted into culture medium by GBS (5).

Type II strains bearing the c protein are more resistant than protein-negative type II GBS to killing by human polymorphonuclear leukocytes (3, 20, 21). In studies of IgA binding as a possible virulence factor for type II GBS, we made the quantitative and qualitative observations described below.

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MATERIALS AND METHODS

Bacterial strains. Most of the strains which we used have been described previously (12, 20). Strain 78-471 is a type II strain with both the TR (α) and TS (β) components of the c protein; thus, the serotype of this strain is II/TR+TS. Strain 79-413 is a type II strain with the TR component (serotype II/TR); strain 80-277 is a type II strain with the TS component (serotype II/TS); and strain 79-176 is a type II strain with no protein identified (serotype II). Strain 80-042 has no identifiable type polysaccharide, but contains both protein components of c protein (serotype NT/TR+TS).

An overnight broth culture in Todd-Hewitt broth started from stock GBS frozen in logarithmic phase was used as a 10% inoculum for 100 ml of fresh broth; this preparation was incubated at 37°C to late logarithmic phase (approximately 4 h). The bacteria were washed in Tris buffer (0.2 M, pH 7.4) supplemented with 0.1% Tween 20 (Fisher Scientific Co., Fair Lawn, N.J.) (Tris-Tween) and then suspended in 2 ml of Tris-Tween. The concentrated suspension of GBS was sonicated on ice by using two 15-s bursts with a Sonifier 200 cell disruptor (Branson Sonic Power Co., Danbury, Conn.) with an output setting of 2 to 3. Bacteria were counted before and after sonication in a Petroff-Hausser chamber, and the results were confirmed with viable counts. Chamber counts and the number of colony-forming units per milliliter increased in parallel after sonication. Microscopy with the Petroff-Hausser chamber and after Gram straining showed that after sonication, ≥80% of the bacterial units were single cocci and most of the remainder were diplococci.

Immunoglobulin preparations and assays. Human myeloma IgA was purchased from Cappel Laboratories, (Organon Teknika, Westchester, Pa.). IgA1 was separated from normal human serum and from myeloma IgA by the method of Roque-Berreira and Campos-Neto (22), using jacalin immobilized on 6% agarose beads (Pierce Chemical Co.,

Rockford, Ill.). The serum was diluted with an equal volume of 0.01 M phosphate-buffered saline (pH 7.4) (PBS), or the myeloma IgA was diluted in PBS to a concentration of 0.5 mg/ml and added to 2-ml columns of immobilized jacalin. IgA1 was eluted with 0.1 M melibiose in distilled water and dialyzed against PBS. The protein content was measured by using the Coomassie blue method (Bio-Rad Laboratories, Richmond, Calif.). Purified IgA2 from a myeloma patient was generously provided by D. C. Heiner. Affinity-purified human IgG was purchased from Cappel Laboratories.

Murine monoclonal antibodies to human IgA1 and IgA2 and murine polyclonal antibody to human IgA were purchased from Southern Biotechnology Associates, Inc., Birmingham, Ala.; all three antibodies were IgG1. Goat anti-mouse IgG (Southern Biotechnology Associates) and goat anti-human IgA (Cappel Laboratories) were conjugated with bovine intestinal alkaline phosphatase by using the one-step glutaraldehyde procedure (2).

The identities of IgA subclasses were determined with an enzyme-linked immunosorbent assay (ELISA) by using a modification of the method of Gregory et al. (10). A 100-ng portion of the appropriate immunoglobulin in 100 μl of 0.1 M carbonate buffer (pH 9.6) was added in duplicate to the wells of Immulon I Removawell plates (Dynatech Laboratories, Inc., Alexandria, Va.) and allowed to adhere for 2 h at 37°C. After three washes with PBS containing 0.05% Tween 20 (PBS-Tween), a predetermined dilution of murine antibody in PBS-Tween was added, the preparation was incubated for 1 h at 37°C. The wells were emptied, washed three times, and filled with alkaline phosphatase-conjugated anti-mouse IgG (diluted 1:2,000 in PBS-Tween), and the preparations were incubated at 37°C for 1 h and at 4°C overnight. After three more washes, *p*-nitrophenyl phosphate (1 mg/ml in diethanolamine buffer) was added, and the plate was incubated in the dark for 30 min at room temperature. A_{405} was measured with an automatic reader (Flow Laboratories, Inc., Irvine, Calif.). In some experiments, conjugated goat anti-human IgA was added to wells coated with IgA preparations, and this was followed by the addition of phosphate substrate as described above.

Binding of ^{125}I-labeled IgA. The methods used for the ^{125}I-IgA binding experiments were modified from the methods described by Russell-Jones et al. (23). Myeloma IgA and purified IgA1 were diluted in PBS and labeled with ^{125}I (Amersham Corp., Arlington Heights, Ill.) to a mean specific activity of 5.5×10^5 cpm/μg by using Iodo-Beads (17) obtained from Pierce Chemical Co. For comparing IgA binding among different GBS strains, 4×10^8 sonicated streptococci (Petroff-Hauser chamber count) were first incubated for 1 h at 25°C with a 100-fold excess unlabeled IgA (relative to ^{125}I-IgA) or with buffer in 1.5-ml conical polypropylene tubes (Research Products International Corp., Mount Prospect, Ill.). Different quantities of ^{125}I-IgA were then added to the GBS for 1 h in a total volume of 300 μl of Tris-Tween. The GBS were pelleted by centrifugation at 10,000 rpm for 4 min in a model 202MK microcentrifuge (Sigma Lab Centrifuges, Osterode am Harz, Federal Republic of Germany), washed twice with Tris-Tween, and counted with a model Biogamma II spectrometer (Beckman Instruments, Inc., Fullerton, Calif.). The conditions necessary for optimal binding were assessed similarly with a single strain (strain 78-471), except that IgA was added to 10^8 GBS cells in 100 μl. In the experiments used for Scratchard analysis, larger amounts of IgA were added to 10^8 GBS cells (strain 78-471) in a volume of 1 ml.

Binding of IgA in an ELISA-filtration system. We also determined the binding of IgA by using an ELISA-filtration system. This more qualitative method had the following advantages compared with the assay for ^{125}I-IgA binding to GBS in polypropylene tubes: radioactive isotopes were not required, smaller amounts of reagents were needed, and exhaustive washing of GBS was simpler. We used a 96-well Millititer GV filtration plate equipped with hydrophilic membranes (pore size, 0.2 μm) that was fitted over a vacuum manifold (Millipore Corp., Bedford, Mass.). The wells and nonfilterable contents were washed by using repeated cycles of filling and vacuum filtration. Additions were in 100-μl volumes of PBS-Tween, and washes were with 200-μl portions of PBS-Tween. The wells were first prewashed and then "blocked" with 200 μl of 2% bovine serum albumin for 30 min at room temperature. After another wash, 5×10^8 GBS cells per ml (harvested from a logarithmic-phase culture, sonicated, and counted in the Petroff-Hauser chamber, as described above) were added and washed. The wells were filled with IgA in 1% bovine serum albumin, incubated at room temperature for 2 h, drained, and washed 10 times. Goat anti-human IgA conjugated with alkaline phosphatase was added for 2 h at 37°C. After 10 more washes, *p*-nitrophenyl phosphate substrate was added, and the plate was incubated in the dark as described above. At this point, the trap in the manifold was replaced with a 96-well microdilution polypropylene plate (Corning Glass Works, Corning, N.Y.), the vacuum was turned on to collect the contents of the corresponding wells of the filtration plate, and A_{405} was measured.

Statistical methods. Specific binding of ^{125}I-IgA was computed by subtracting the number of counts per minute bound in presence of an excess of unlabeled IgA (nonspecific binding) from the number of counts per minute bound in

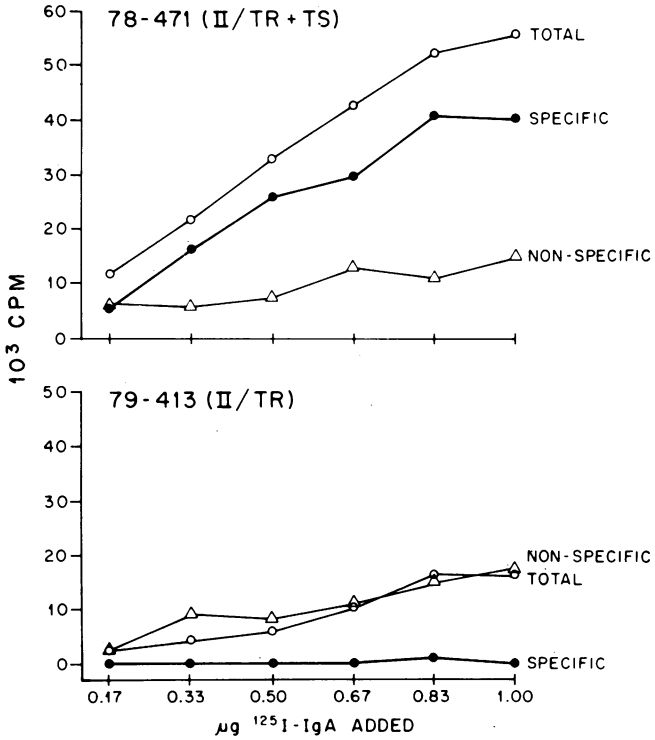

FIG. 1. Binding of ^{125}I-IgA (myeloma) to two GBS strains when different amounts were incubated with 4×10^8 streptococci in a volume of 300 μl.

TABLE 1. Specific binding of human myeloma IgA to GBS strains

Strain (serotype)	No. of expts	Amt (ng) of IgA bound when the following amt of ^{125}I-IgA were added[a]:						
		0.17 μg	0.33 μg	0.50 μg	0.67 μg	0.83 μg	1.00 μg	1.17 μg[b]
78-471 (II/TR+TS)	5	9.02 ± 5.95	14.70 ± 7.17	25.25 ± 9.98	30.15 ± 13.42	42.91 ± 14.72	42.46 ± 14.95	34.94 ± 11.51
80-277 (II/TS)	5	4.84 ± 4.56	8.60 ± 5.63	14.32 ± 7.94	20.57 ± 17.00	35.48 ± 17.87	40.11 ± 11.65	42.28 ± 10.97
80-042 (NT/TR+TS)	3	4.50 ± 3.13	9.34 ± 8.80	10.50 ± 6.71	20.60 ± 3.79	23.32 ± 15.65	24.62 ± 24.52	18.45 ± 2.30
79-413 (II/TR)	5	0.30 ± 0.63[c]	0.11 ± 0.24[d]	0.84 ± 1.89[d,e]	1.80 ± 2.05[d]	0.70 ± 1.06[d,f]	6.18 ± 6.93[d,f]	0[c,e]
79-176 (II)	5	1.95 ± 2.37	0.20 ± 0.44[d]	1.46 ± 3.26[d]	2.51 ± 4.19[d]	6.41 ± 8.04[d,e]	2.81 ± 5.79[d,f]	0[c,e]

[a] Amount of IgA bound (mean ± standard deviation) in a total volume of 300 μl.
[b] This amount of ^{125}I-IgA was added to each strain in only two experiments.
[c] $P < 0.05$ relative to strain 78-471 (based on the Bonferroni test [19]).
[d] $P < 0.01$ relative to strain 78-471 (based on the Bonferroni test [19]).
[e] $P < 0.05$ relative to strain 80-277 (based on the Bonferroni test [19]).
[f] $P < 0.01$ relative to strain 80-277 (based on the Bonferroni test [19]).

absence of unlabeled IgA (total binding). Binding among streptococcal strains was compared by using analysis of variance, and subsequent pairwise comparisons were made by using the Bonferroni test to correct for multiple comparisons (19).

Binding data were transformed for Scratchard analysis with a computer software program developed by McPherson (18) and distributed by Elsevier-Biosoft, Cambridge, England, which used nonlinear, least-squares, curve-fitting techniques.

RESULTS

Demonstration of specific binding of ^{125}I-IgA by GBS. Figure 1 shows the binding of human myeloma IgA by a pair of type II GBS, strain 78-471, which has both the TR (α) and TS (β) components of the c protein, and strain 79-413, which has only the TR (α) component. The total binding (counts per minute bound in the absence of unlabeled IgA) increased for both strains with the addition of more ^{125}I-IgA, but was three to five times greater for the type II/TR+TS strain than for the II/TR strain at all concentrations of ^{125}I-IgA. The levels of nonspecific binding of IgA (counts per minute bound in the presence of an excess of unlabeled IgA) were very similar for the type II/TR+TS strain and the II/TR strain and accounted for essentially all of the counts per minute bound to the latter strain. The level of specific binding (total binding minus nonspecific binding) was negligible for the type II/TR streptococcus, but the level of specific binding of IgA to the type II/TR+TS strain increased in a dose-dependent manner.

Comparison of specific binding of ^{125}I-IgA in different strains of GBS. To compare data from different experiments, the levels of specific binding were calculated and expressed as nanograms of ^{125}I-IgA bound to 4×10^8 streptococcal units. Composite data from five experiments (Table 1) segregated the five type II GBS strains into two distinct groups. The three strains which had the TS (β) component (type II/TR+TS, II/TS, and NT/TR+TS strains) exhibited a dose-dependent increase in specific binding as more ^{125}I-IgA was added. The differences in the levels of specific binding of IgA for the three TS-bearing strains were not statistically significant. In contrast, the two strains which lacked the TS (β) component (type II/TR and II strains) showed significantly less binding than the type II/TR+TS strain at essentially all concentrations of ^{125}I-IgA added and did not demonstrate a progressive or consistent increase in the level of specific binding with increasing amounts of ^{125}I-IgA added (Table 1).

Specific binding of myeloma IgA and normal serum IgA1 to GBS. Because we could not directly distinguish the binding of ^{125}I-IgA by the Fc domain from the binding of IgA antibody by Fab sites, we performed the experiment summarized in Table 2. ^{125}I-labeled IgA from myeloma serum and labeled IgA1 purified from normal serum did not differ significantly in their binding to type II/TR+TS GBS. This indicates that binding was not dependent on the immunological specificity of the IgA antibody.

Conditions for optimal binding. Table 3 shows the influence of different temperatures on IgA binding to type II/TR+TS GBS during 24 h of incubation. Specific binding was clearly more rapid at room temperature than at 0 or 37°C. The levels of non-specific binding did not differ appreciably at the three temperatures evaluated.

When type II/TR+TS GBS were heat killed (90°C for 1 h) prior to the addition of ^{125}I-IgA, the level of total binding was reduced by 85 to 90% compared with binding by unheated, viable streptococci (Fig. 2). Although specific binding was not measured with excess, unlabeled IgA in this experiment, this preparation of ^{125}I-IgA exhibited minimal nonspecific binding in other experiments (Table 3). Therefore, it seems clear that heat treatment of GBS reduced the specific binding capacity substantially.

Since results of the experiments described above were ambiguous regarding the time that IgA binding at 25°C reached equilibrium, additional studies were done. These

TABLE 2. Comparison of specific binding of myeloma IgA and normal IgA1 to type II/TR+TS GBS

IgA prepn	Amt (ng) of IgA bound when the following amt of ^{125}I-IgA were added[a]:						
	0.17 μg	0.33 μg	0.50 μg	0.67 μg	0.83 μg	1.00 μg	1.17 μg
Myeloma	8.10 ± 0.64	16.32 ± 0.64	25.35 ± 3.74	35.56 ± 0.86	40.60 ± 5.61	49.50 ± 5.04	46.21 ± 4.74
Normal	12.79 ± 2.74	26.27 ± 8.70	31.39 ± 2.25	45.16 ± 8.94	43.69 ± 11.56	45.91 ± 16.86	57.84 ± 6.50

[a] Amount of IgA bound (mean ± standard deviation) to 4×10^8 strain 48-471 cells in a total volume of 300 μl. The values are based on the results of three experiments.

TABLE 3. Effect of temperature on IgA binding to GBS

Length of incubation (h)	IgA binding (cpm)[a]								
	0°C			25°C			37°C		
	Total	Nonspecific	Specific	Total	Nonspecific	Specific	Total	Nonspecific	Specific
2	29,836	1,337	28,499	52,582	1,388	51,194	42,766	1,716	41,050
4	34,354	1,140	33,214	52,162	1,173	50,989	51,042	1,808	49,234
6	42,293	982	41,311	55,981	1,321	54,660	47,699	1,877	45,822
24	53,409	1,129	52,280	63,711	1,373	62,338	51,820	2,705	49,115

[a] Amount of IgA bound when 3 μg of ^{125}I-IgA and 10^8 GBS cells (type II/TR+TS strain 78-471) in 100 μl of Tris-Tween were incubated for different times and at different temperatures. Some preparations had been preincubated with 300 μg of unlabeled IgA for 1 h at the temperature tested. Determinations of viable counts at 0 and 24 h showed that the GBS did not multiply under any of the conditions tested.

showed that equilibrium was attained at 6 to 8 h and that the amounts of IgA bound did not change over the subsequent 24 h (data not shown).

Reversibility of ^{125}I-IgA binding. The addition of a 1,000-fold excess of unlabeled IgA to 10^8 type II/TR+TS GBS cells which had been incubated for 2 h with ^{125}I-IgA resulted in progressive displacement of the radioactivity bound to streptococci (>60% reduction at 67 h) (Fig. 3). In contrast, in the control tubes (no unlabeled IgA added), binding of ^{125}I-IgA continued to increase for 6 h and then was essentially unchanged for 3 days.

Quantitative findings. The incubation of type II/TR+TS GBS with different quantities of ^{125}I-IgA for 24 h at room temperature gave the results shown in Fig. 4B. The level of specific binding increased with the addition of increasing amounts of ^{125}I-IgA and appeared to plateau around 24 μg of ^{125}I-IgA. These data were transformed for the Scratchard plot shown in Fig. 4A. Extension of the line to the abscissa, representing the theoretical point at which all GBS binding sites would be saturated, suggested a maximal binding of 26.9×10^{-10} M IgA to 10^8 streptococci, which is equivalent to 1.6×10^4 molecules of IgA per cell of this strain under these conditions. The derived dissociation constant was 19.3×10^{-9} M. Similarly, the specific binding capacity calculated from a saturating value of 24 μg of IgA per 10^8 streptococci (Fig. 4B) was 3.76×10^4 molecules of IgA per streptococcus.

Binding of IgA1 and IgA2. To determine the subclass composition of the myeloma IgA used in most of the experiments described above, an ELISA (Table 4) was carried out. As expected, polyclonal murine antibody to human IgA reacted with myeloma IgA, IgA1, and IgA2, but not with the controls. Monoclonal anti-IgA1 bound to myeloma IgA and IgA1, but not to IgA2. Monoclonal antibody to IgA2 reacted significantly only with the IgA2 preparation (Table 4). We concluded from these results that the myeloma IgA consisted of IgA1 and no detectable IgA2.

We next used the ELISA-filtration assay to evaluate the binding of IgA and its subclasses to GBS. GBS in filtration wells were first incubated with IgA preparations at three concentrations and then with enzyme-conjugated goat anti-

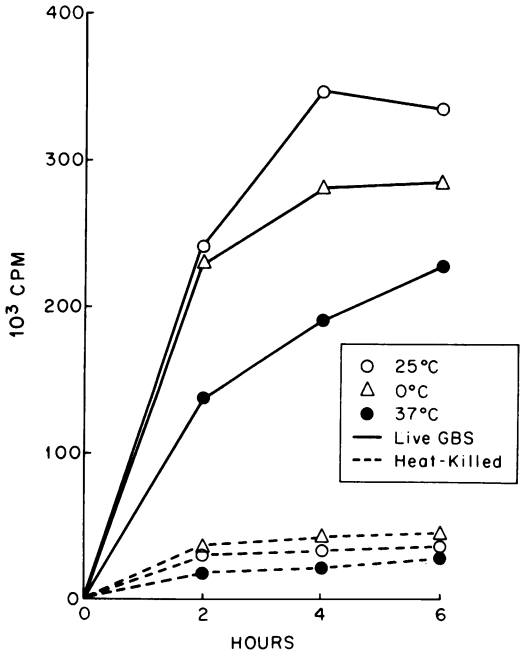

FIG. 2. Effect of heat killing GBS (type II/TR+TS strain 78-471) on total binding of ^{125}I-IgA (myeloma) at three temperatures. For each experiment 5 μg of ^{125}I-IgA was incubated with 10^8 streptococci in a volume of 100 μl.

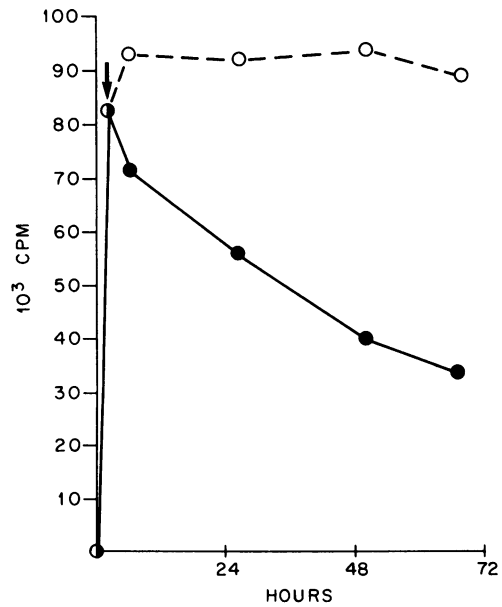

FIG. 3. Displacement of ^{125}I-IgA (myeloma) from GBS (type II/TR+TS strain 78-471)'by excess unlabeled IgA. For each experiment 3 μg of ^{125}I-IgA was incubated with 10^8 GBS cells in a volume of 100 μl. At 2 h (arrow), 3 mg of unlabeled IgA in 300 μl was added to some tubes (●), and 300 μl of Tris-Tween was added to others (○).

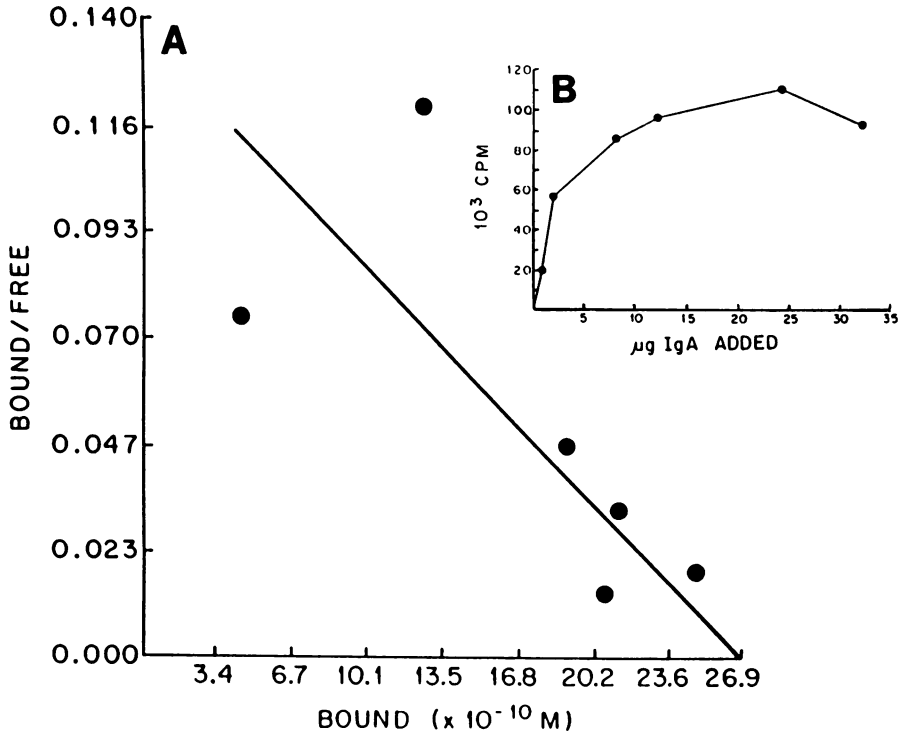

FIG. 4. (A) Scratchard plot of ^{125}I-IgA (myeloma) binding by strain 78-471 (type II/TR+TS). $r = 0.728$. The plot was derived from the binding data shown in panel B. After preincubation of 10^8 streptococci with a 100-fold excess of unlabeled IgA, ^{125}I-IgA was added at room temperature in a volume of 1 ml for 24 h. The maximal binding of Iga to 10^8 GBS cells was 26.9×10^{-10} M (16,000 molecules per streptococcus). The dissociation constant was 19.3 nM.

human IgA that recognized IgA1 and IgA2 equally in the ELISA (data not shown). As Table 5 shows, the absorbance values subsequently developed with the phosphate substrate were similar for the protein-negative type II strain and for wells containing no bacteria. In contrast, the type II/TR+TS strain clearly bound myeloma IgA (IgA1), purified IgA1, and IgA2. In separate experiments, we showed that, under the conditions of this assay, the binding of IgA1 to this type II/TR+TS GBS was saturated at approximately 0.04 μg of IgA per well (data not shown), thus explaining the lack of a dose response in absorbance readings with larger quantities of myeloma IgA and purified IgA1. Although the absorbance readings for IgA2 binding to this strain were lower than those for IgA1 binding, they were distinctly above the background level and above the readings for protein-negative GBS and demonstrated a dose response. Similar results were found when these two strains in filtration wells were incubated with IgA1 and IgA2, then with the corresponding murine monoclonal antibodies, and finally with conjugated goat anti-mouse IgA (data not show). These findings indicate that the type II/TR+TS strain bound both IgA1 and IgA2 and suggest

TABLE 4. Identification of IgA subclasses by ELISA

Immunoglobulin prepn	A_{405} after the addition of the following murine antibodies[a]:		
	Anti-IgA	Anti-IgA1	Anti-IgA2
Myeloma IgA	1.33	1.50	0.14
IgA1[b]	1.33	1.36	0.11
IgA2	1.07	0.17	1.50
IgG	0	0.02	0.03
None	0	0.01	0

[a] A_{405} values after successive additions to wells of (i) 100 ng of immunoglobulin, (ii) polyclonal (anti-IgA) or monoclonal (anti-IgA1, anti-IgA2) murine antibody, (iii) goat anti-mouse IgA conjugated with alkaline phosphate, and (iv) phosphate substrate. See Materials and Methods.
[b] Purified from myeloma IgA on jacalin-Sepharose.

TABLE 5. Binding of IgA subclasses as determined by the ELISA-filtration assay

Strain (serotype)	A_{405} after the addition of the following IgA prepn[a]:			
	Amt of IgA added (μg/well)	Myeloma IgA	IgA1[b]	IgA2
79-176 (II)	5.0	0.45	0.35	0.09
	1.0	0.33	0.11	0.02
	0.2	0.13	0.07	0.06
	0	0	0.03	0
78-471 (II/TR+TS)	5.0	1.08	1.54	0.72
	1.0	0.99	1.37	0.21
	0.2	1.00	1.41	0.12
	0	0.01	0.03	0.02
None	5.0	0.40	0.27	0.05
	1.0	0.28	0.08	0
	0.2	0.11	0.05	0.01
	0	0.08	0.10	0.11

[a] A_{405} values after successive additions to wells containing 5×10^7 GBS cells of (i) the IgA preparation, (ii) alkaline phosphatase-conjugated goat anti-human IgA, and (iii) phosphate substrate. See Materials and Methods.
[b] Purified from myeloma IgA on Jacalin-Sepharose.

that the dynamics of binding for the two subclasses may differ.

DISCUSSION

In their original description of the nonimmune interaction between human IgA and GBS, Russell-Jones et al. demonstrated the binding of radioiodinated human IgA to a 130,000-molecular-weight protein isolated from GBS, which was presumably the TS (β) component of the c protein. ^{125}I-IgA binding to GBS could be inhibited by Fc, but not Fab fragments of IgA (23). Cleat and Timmis cloned the TR (α) and TS (β) genes of a type Ia/c GBS in *Escherichia coli* and also demonstrated ^{125}I-IgA binding to a protein identified as the TS (β) component (7). Studying the reaction of IgA Fc preparations with intact GBS and their secreted proteins, Brady and Boyle found few exceptions to the relationship between binding of IgA and expression of the TS (β) antigen (5). In our study of a small number of type II strains, we found that the inhibitable, dose-dependent reaction with ^{125}I-IgA occurred only when the TS (β) component was present and could be largely reversed by the addition of excess unlabeled IgA. The fact that very similar amounts of myeloma IgA and purified normal IgA1 combined with GBS indicates that binding was not related to the antibody specificity of the IgA.

IgA binding to GBS was signficantly more rapid at room temperature than at 0 or 37°C. Heating of streptococci (90°C for 1 h) reduced the capacity of the cells to combine with IgA by 80 to 90%. Brady and Boyle demonstrated IgA binding to hot acid extracts of GBS containing the TS (β) protein (5). Therefore, it seems more likely that heating removes the protein from GBS or modifies its attachment to the cell wall than that the IgA-binding site of the TS (β) protein is heat labile.

The best-studied bacterial receptor for immunoglobulins is staphylococcal protein A. Besides the classical Fc-combining activity, protein A is also capable of binding Fab sites, which may explain the nonimmune reaction of protein A with immunoglobulins other than IgG (9). Hemolytic streptococci belonging to several Lancefield groups also bind IgG (14), evidently through receptors for both Fc and Fab sites (8). Except for the report of Jurgens et al. that the CAMP factor may interact with the Fc of IgG and IgM (13), GBS have not been included in reports of streptococcal binding of IgG.

Since the extent of chain formation can vary with different streptococcal strains, we compared equivalent numbers of bacterial cells in quantitative binding studies. This could be consistently accomplished with brief sonication, which reduced streptococcal chains to ≥80% single cocci. When known numbers of streptococci prepared in this way were reacted with graded amounts of ^{125}I-IgA under optimal conditions for binding, the results of a Scratchard analysis suggested that a single bacterium of the strain studied (strain 78-471) had a capacity of approximately 16,000 molecules of monomeric, serum IgA. This figure can be compared with 80,000 molecules of IgG per straphylococcus, the estimate of Kronvall et al. for the capacity of the Cowan I strain of *Staphylococcus aureus* (15).

ELISA analysis with monoclonal antibodies to the IgA subclasses demonstrated that the myeloma IgA used in most of our studies was exclusively IgA1. Therefore, the quantitative data presented here may be applicable only to GBS binding of IgA1. Using an ELISA-filtration assay which was considerably less costly than the ^{125}I-IgA binding studies, we showed that there was significant binding of the myeloma IgA to a type II strain bearing the c protein (type II/TR+TS strain), but not to a c-protein-negative type II GBS. Moreover, this assay demonstrated that there was GBS binding of both IgA1 and IgA2. This is consistent with the data of Russell-Jones et al., who reported inhibition of ^{125}I-IgA binding to GBS with unlabeled IgA2 (23). Brady and Boyle also found that IgA1 and IgA2 bound to the surfaces of TS (β)-positive GBS. However, these authors demonstrated binding of secretory IgA only to the TS (β) component in culture supernatants and not to intact GBS (5).

The c protein of GBS may play a significant role in some infections. For example, it has been reported recently that the c protein may be more common in GBS associated with early-onset neonatal infections (C. Chun, E. M. Ayoub, L. J. Brady, M. P. Boyle, and H. Dillon, Pediatr. Res. **25**:175A, 1989). Rabbit antibody to the c protein partially protects mice challenged with type Ib and Ia/c GBS (16) and is opsonic for the same serotypes (1). Recent observations that type II strains with the c protein resist killing by human granulocytes (3, 20, 21) and ingestion by human monocytes and macrophages (N. R. Payne, N. F. Concepcion, and B. F. Anthony, Pediatr. Res. **21**:419A, 1987) more effectively than protein-negative type II strains suggest that this protein may be a virulence factor. Whether IgA binding to the c protein contributes directly to the pathogenicity of GBS bearing this protein on their surfaces or whether the c protein is related to virulence in another way will require further study.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-14827 and AI-26257 from the National Institute of Allergy and Infectious Diseases.

We thank Patricia Ferrieri for the streptococcal strains, DeAnna Lopez for technical assistance, Chung-Yin Chiu for statistical consultation, James Padbury for advice concerning the Scratchard analysis, and Douglas Heiner for the gift of the IgA2.

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