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Comparison of Albumin Receptors Expressed on Bovine and Human Group G Streptococci

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The albumin receptor expressed by bovine group G streptococci was extracted and affinity purified. The protein was characterized for species reactivity, and monospecific antibodies were prepared to the purified receptor. The bovine group G albumin receptor was compared functionally, antigenically, and for DNA homology with the albumin-binding protein expressed by human group G streptococci. In agreement with previous reports, the albumin-binding activity of human strains was mediated by a unique domain of the type III immunoglobulin G-Fc-binding molecule, protein G. The albumin receptor expressed by bovine group G strains was found to lack any immunoglobulin G-binding potential but displayed a wider profile of species albumin reactivity than protein G. Both albumin receptors could inhibit the binding of the other to immobilized human serum albumin, and each displayed similar binding properties. Antigenic comparison of the two albumin receptors demonstrated a low level of cross-reactivity; however comparison at the DNA level, using an oligonucleotide probe specific for the albumin-binding region of protein G, demonstrated that the two albumin receptors expressed by human and bovine group G streptococcal strains do not display significant homology.

Receptors for a number of serum proteins including albumin (16, 30–33), immunoglobulin (2, 5, 18, 25, 35), plasmin (15), fibrinogen (13), β_2-microglobulin (12), C1q (34), and kininogen (28) have been recognized on certain gram-positive bacteria. The immunoglobulin-Fc-binding proteins have been studied extensively because of their practical value for immunochemical applications (6, 7, 10, 22, 25). Analysis of the type III Fc-binding protein (more frequently designated streptococcal protein G) associated with the majority of human group C and group G streptococcal isolates has revealed a number of interesting properties. This molecule, in addition to binding a wide species range of immunoglobulin Fc fragments, has been shown to bind certain species of albumin, $F(ab')_2$ fragments, α_2-macroglobulin, and kininogen (28). These properties appear to be mediated by distinct portions of the protein G molecule (4, 26, 27). The albumin-binding region and the immunoglobulin G (IgG)-Fc-binding portion of the protein have been separated by chemical methods and by recombinant DNA technology (2, 4, 26, 27) and shown to reside at the N-terminal and central portions of the protein G molecule, respectively.

Certain bovine group G streptococci have been reported to express a receptor for albumin (16, 30–33), in addition to expressing a type IV Fc-binding protein that shows very limited species reactivity (17, 19, 24). The finding that human group G streptococci express a single molecule, protein G, that can act as both an albumin receptor and an Fc-binding protein prompted us to test the possibility that the albumin receptors expressed on bovine and human group G isolates were related. The studies described in this report were designed to compare the albumin receptors expressed on bovine and human group G isolates functionally, antigenically, and at the DNA level.

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MATERIALS AND METHODS

Bacteria. Seven bovine group G beta-hemolytic streptococcal isolates obtained from the Quality Milk Promotion Services Laboratory at the New York State College of Veterinary Medicine, Cornell University, Ithaca, were used in this study. The bacteria were grown overnight at 37°C as stationary cultures in Todd-Hewitt broth.

Proteins. The various species of albumin used in this study were obtained from Sigma Chemical Co., St. Louis, Mo. Rabbit Fc fragments were obtained from Jackson Immunoresearch Laboratories, West Grove, Pa. For affinity purification of the bacterial albumin receptors, human serum albumin (HSA) was immobilized onto the high-capacity support Affi-Prep-10 (Bio-Rad Laboratories, Richmond, Calif.) as described previously (22). Wild-type protein G was isolated from a group G streptococcus as described previously (21).

Iodination of proteins. The HSA or rabbit Fc fragments were iodinated by the mild lactoperoxidase method, using Enzymobeads (Bio-Rad), as described previously (20). Labeled proteins were separated from free iodine by passage over a G25 column (PD-10; Pharmacia, Piscataway, N.J.) and collected in 10 mM Veronal-buffered saline (VBS) (pH 7.35) containing 0.001 M Mg^{2+}, 0.00015 M Ca^{2+}, 0.1% gelatin, and 0.02% NaN_3 (VBS-gel). The labeled proteins routinely had specific activities of approximately 0.3 mCi/mg.

Determination of binding of radiolabeled proteins to bacteria. The beta-hemolytic bovine group G streptococci were grown in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) overnight at 37°C as stationary cultures. The bacteria were harvested by centrifugation and suspended in VBS-gel. The optical density at 550 nm was determined to standardize the concentration of organisms used in subsequent tests. An optical density of 0.3 at 550 nm corresponded to approximately 2×10^9 organisms per ml (36). A standard number of bacteria (approximately 10^9 organisms) were incubated with labeled proteins (approximately 30,000 cpm per tube) in a total volume of 500 μl of VBS-gel for 1 h at 37°C. The

bacteria were pelleted by centrifugation at 1,000 × g for 10 min and washed twice with 2 ml of 10 mM VBS containing 0.01 M EDTA and 0.1% gelatin, (pH 7.35) (EDTA-gel). The radioactivity associated with the bacteria was determined in a Beckman 5500 Autogamma Counter (Beckman Instruments, Inc., Fullerton, Calif.). All estimates were carried out in duplicate.

Extraction of albumin receptors. The colony-selected group G streptococcal strain BG5-AB3 was grown overnight at 37°C in Todd-Hewitt broth. Approximately 6 g (wet weight) of bacteria was recovered from 3 liters of Todd-Hewitt broth. Bacteria were extracted with hot acid or hot alkali or by heat extraction or detergent treatment. The conditions used for each of these extraction procedures have been described in detail previously (35).

Dot-blotting procedure. Dot blotting was performed with the Bio-Rad Bio-Dot microfiltration apparatus and a modification of the Bio-Rad procedure as described previously (34). A piece of nitrocellulose previously soaked in 20 mM Tris–500 mM NaCl (pH 7.5) (TBS) was placed in the apparatus. A 100-μl aliquot of each column fraction was added to each well and incubated for 1 h at ambient temperatures. Each well was washed with TBS containing 0.05% Tween 20, and the nitrocellulose was removed and washed four times in VBS (pH 7.35) containing 0.15% Tween 20 and 0.50% gelatin (VBS-T-gel). Each wash was carried out for 15 min using 250 ml of buffer. The nitrocellulose was then probed for 3 h in the VBS-T-gel buffer containing 2 × 10^5 cpm of the appropriate ^{125}I-labeled protein per ml. After being probed, the nitrocellulose was washed four times in 0.01 M EDTA–1 M NaCl–0.25% gelatin–0.15% Tween 20 (15 min for each wash) and allowed to air dry. All washing and probing steps were carried out at ambient temperature. Autoradiographs were prepared from the nitrocellulose blots by exposing them to Kodak XAR-5 film with an intensifying screen for various times at −70°C before photographic development.

Polyacrylamide gel electrophoresis and Western blotting (immunoblotting) procedure to detect albumin-binding proteins. Protein samples were denatured by boiling for 5 min in 2% (wt/vol) sodium dodecyl sulfate (SDS)–5% (vol/vol) β-mercaptoethanol–10% (vol/vol) glycerol–0.01% (wt/vol) bromphenol blue–0.5 M Tris-HCl (pH 6.8). Denatured proteins were electrophoresed on duplicate 10% polyacrylamide slab gels at 5 mA per slab for 16 h by the method of Laemmli (14). Proteins were visualized on one of the gels by staining with Coomassie brilliant blue. Molecular weight standards (Sigma) α_2-macroglobulin (180,000), β-galactosidase (116,000), fructose-6-phosphate (84,000), pyruvate kinase (58,000), furinase (48,500), lactic dehydrogenase (36,500), and triosephosphate isomerase (26,600) were included in each SDS-polyacrylamide gel assay.

The proteins on the duplicate gel were electrophoretically transferred to nitrocellulose (Bio-Rad) by a modification of the method described by Towbin et al. (29). Briefly, SDS slab gels were presoaked for 30 min in 25 mM Tris–192 mM glycine–20% methanol (pH 8.3), assembled into the high-field-intensity Transblot system (Bio-Rad), and electrophoresed in that buffer at 70 V for 3 h.

The nitrocellulose blots were washed four times with 250 ml of 10 mM VBS (pH 7.4) containing 0.25% gelatin and 0.25% Tween 20 (VBS-T-gel) to saturate the remaining protein-binding sites on the nitrocellulose. Each wash was carried out for 15 min at ambient temperature. The nitrocellulose membranes were incubated for 3 h at ambient temperature in 25 ml of VBS-T-gel containing 2 × 10^5 cpm of ^{125}I-labeled HSA or ^{125}I-labeled rabbit IgG Fc fragments per ml. The nitrocellulose membranes were then washed four times (for 15 min each time) with 250 ml of VBS containing 0.01 M EDTA, 1 M NaCl, 0.25% gelatin, and 0.25% Tween 20 on a rocking platform at ambient temperature. The membranes were dried and autoradiographed by using Kodak XAR-5 film and Kodak X-Omatic intensifying screens at −70°C for 1 to 3 days.

Chicken anti-albumin receptor antibody. Monospecific antibodies were prepared in chickens by using the affinity-purified albumin-binding receptor as immunogen. The immunization protocol followed that previously used to prepare antibodies to bacterial Fc-binding proteins (23). Essentially, the chicken was injected with an immunogen consisting of approximately 50 μg of albumin receptor intramuscularly or subcutaneously in complete Freund's adjuvant. The immunogen used was a single form of the albumin receptor protein (M_r, 45,000) that was isolated first by affinity purification by binding to and elution from a column of immobilized HSA and then further purified by SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to nitrocellulose by electroblotting, and a band corresponding to the 45,000-M_r albumin receptor activity was cut from the blot. The nitrocellulose strip containing the antigen was solubilized by treatment with dimethyl sulfoxide, emulsified in adjuvant, and used as the immunogen as described previously (1). The chickens were allowed to rest for 3 weeks and then injected with approximately 50 μg of the immunogen, prepared as described above, that had been emulsified in incomplete Freund's adjuvant. Eggs were collected from the chickens, immunoglobulins were extracted as described previously (3), and the production of antibody was monitored by the ability of yolk extracts to inhibit binding of ^{125}I-labeled affinity-purified albumin receptors to immobilized HSA.

DNA dot blot hybridization. The dot blot was performed with a Bio-Dot microfiltration apparatus (Bio-Rad). Chromosomal DNA was isolated from streptococci as described previously (19). DNA samples were completely denatured by the addition of 100 μl of 0.4 M NaOH–10 mM EDTA and subsequent heating to 100°C for 10 min. Following neutralization with an equal volume of ice-cold 2 M ammonium acetate (pH 7.0), DNA was applied to an Opti-bind membrane (Schleicher & Schuell, Inc., Keene, N.H.) which had been presoaked in 9 mM trisodium citrate–90 mM sodium chloride (pH 7.4). Following vacuum filtration of DNA samples, each well was washed with 3 mM trisodium citrate–30 mM sodium chloride (pH 7.4). The blotted membrane was rinsed with the same solution, and the DNA was baked onto the membrane by heating at 80°C for 1 h. The subsequent hybridization steps were performed as described previously (8), with minor modifications. Prehybridization in the absence of the oligonucleotide probe was carried out at 25°C by incubation with 17 μl of the hybridization solution [4.5 mM trisodium citrate, 45 mM sodium chloride (pH 7.4), 1 mg of heparin per ml, 0.2% sodium PP_i, 0.2% SDS, 100 μg of poly(A) per ml] per cm^2 for 1 h. Following this step, the specific labeled oligonucleotide probe (10^6 cpm/ml of hybridization solution) was added, and hybridization was performed at 25°C for 18 to 24 h. The synthetic oligonucleotide probe (5'-AAATATGGAGTAAGTGACTAT-3') was obtained from Genetic Designs, Inc., Houston, Tex., and corresponds to a conserved sequence present in the albumin-binding domains of the type III bacterial Fc-binding protein, protein G (9, 11).

The probe was end labeled with [^{32}P]ATP, using 21 U of

terminal deoxynucleotydyl transferase as described previously (8). Unincorporated [^{32}P]ATP was removed by using a G25 gel filtration column (PD-10; Pharmacia). Following hybridization, the blotted membrane was washed three times with 200 ml of 4.5 mM trisodium citrate–45 mM sodium chloride (pH 7.4) containing 0.2% SDS at 25°C for 10 min (each wash). A final wash to set the stringency was performed at 49°C (5°C below the calculated denaturation temperature for this particular oligonucleotide) in 1.5 mM trisodium citrate–15 mM sodium chloride (pH 7.4) for 5 min. The reactivity of the probe with the chromosomal DNA preparations was visualized by autoradiography.

Competitive binding radioimmunoassay to detect albumin-binding activity. The competitive binding radioimmunoassay was a modification of the procedure we described previously for quantification of the IgG-binding activities of bacterial immunoglobulin-binding-protein extracts (22). The assay was conducted as follows. Test samples were diluted in VBS-gel, and 1 ml of each dilution was mixed with 0.1 ml of a standard suspension of agarose beads to which HSA had been covalently coupled (Immunobeads; Bio-Rad) and with 0.1 ml of ^{125}I-labeled albumin receptor or ^{125}I-protein G (approximately 300,000 cpm). Tubes were incubated for 60 min at 37°C, at which time 2 ml of EDTA-gel was added, followed by centrifugation at 1,000 × g for 10 min. The supernatant fluid was decanted, and the wash was repeated. Radioactivity which remained associated with the beads was determined with a Beckman Autogamma Counter. The number of counts bound in the presence of different dilutions of unlabeled fluid-phase binding proteins was determined. All estimations were performed in duplicate.

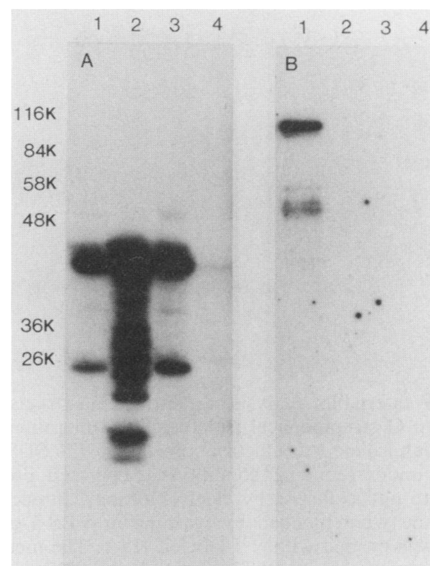

FIG. 1. Western blot analysis of albumin-binding proteins extracted from bovine group G streptococcal strain BG5 AB3 by various procedures. Fifty microliters of each extract was electrophoresed on a 10% SDS–polyacrylamide gel under reducing conditions. Lane 1, Neutral heat extract; lane 2, acid heat extract; lane 3, an alkaline heat extract; lane 4, Triton X-100 extract. The separated proteins were transferred onto nitrocellulose by electroblotting. Unreacted sites on the membrane were blocked by washing in VBS-T-gel, and the membrane was probed with radiolabeled HSA (A) or radiolabeled rabbit Fc fragments (B). The probed membrane was washed and exposed to X-ray film for 48 h at −70°C with intensifying screens.

RESULTS

Expression of albumin receptors on bovine group G streptococci. Seven group G, bovine, beta-hemolytic streptococci and four colony-selected variants of one isolate, BG5 (previously selected for expression of high levels of type IV IgG-binding protein [19]), were screened for their ability to bind HSA. The results obtained demonstrate that all of the bovine group G strains tested could bind ^{125}I-labeled HSA in a dose-dependent manner (Table 1). The protein G-positive human isolate G1400 was included as a positive control, and the group A streptococcal isolate 64/14 was included as a negative control. Each of these bacterial isolates was also tested for binding to ^{125}I-labeled rabbit Fc fragments and, in agreement with results of our previous study (19), only strain BG5 and its colony-selected variants bound rabbit immunoglobulin Fc fragments (data not shown).

Extraction of albumin receptors. A variety of different extraction procedures were compared to determine the optimal method for solubilizing the albumin-binding activity. Heat treatment at neutral, acid, or alkaline pH or treatment of bacteria with the detergent Triton X-100 resulted in the solubilization of albumin-binding activity. A comparison of the different extraction procedures for one strain, BG5 AB3, was carried out by Western blotting analysis (Fig. 1A). The greatest yield and least heterogeneous preparation of albumin-binding receptors was recovered in the neutral heat extract of the bacteria (Fig. 1A). Since BG5 had previously been selected for expression of immunoglobulin-binding activity (19), a parallel blot was probed with ^{125}I-labeled rabbit Fc fragments; the results (Fig. 1B) demonstrate that IgG-binding proteins were solubilized by some, but not all, of the extraction procedures. A comparison of the molecular species responsible for the rabbit IgG-Fc-binding properties and the albumin-binding activity demonstrated that these two functional activities were separate and distinct (compare Fig. 1A and B).

On the basis of these studies it appeared that the optimal method for solubilizing bovine group G albumin receptors was by heat treatment at neutral pH (Fig. 1A). Treatment of any of the albumin-receptor-positive bovine group G streptococcal isolates tested resulted in solubilization of a similar series of albumin-binding proteins, with the predominant binding activity displaying an M_r of approximately 45,000 (Fig. 2). A series of lower-molecular-weight albumin-binding proteins were also detected. Subsequent studies demonstrated that all of the albumin-binding receptors were antigenically related (see below).

Since all of the strains shown in Table 1 displayed a similar profile we concentrated the remainder of our studies on isolating and characterizing the albumin-binding activity from strain BG1. This strain was selected because it displayed high levels of albumin-binding activity and was devoid of any immunoglobulin-binding proteins.

Affinity purification of the albumin receptor from a bovine group G streptococcus. Heat extracts of strain BG1 were affinity purified on a column of immobilized HSA. The heat extract was applied to the HSA column at neutral pH, and unbound proteins were removed by washing with 10 mM phosphate-buffered saline (pH 7.3). When the optical density at 280 nm returned to baseline, bound proteins were eluted by using glycine-HCl (pH 2). All of the fractions were screened, following neutralization, for albumin-binding activity by using the dot blot procedure described in Materials and Methods. All of the albumin-binding activity was found

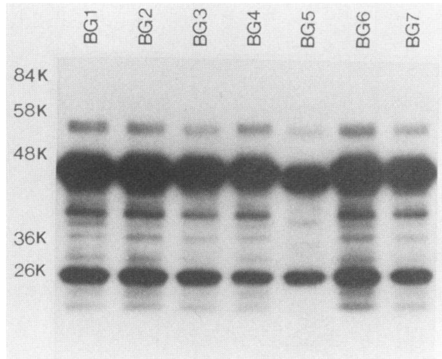

FIG. 2. Western blot analysis of neutral heat extracts from seven bovine group G streptococcal isolates. Fifty microliters of a heat extract of each isolate was electrophoresed on 10% SDS-polyacrylamide gels under reducing conditions. Separated proteins were transferred to nitrocellulose by electroblotting. Unreacted sites on the membrane were blocked by washing in VBS-T-gel, and the membrane was probed with ^{125}I-labeled HSA. The membrane was washed and exposed to X-ray film for 48 h at −70°C with intensifying screens.

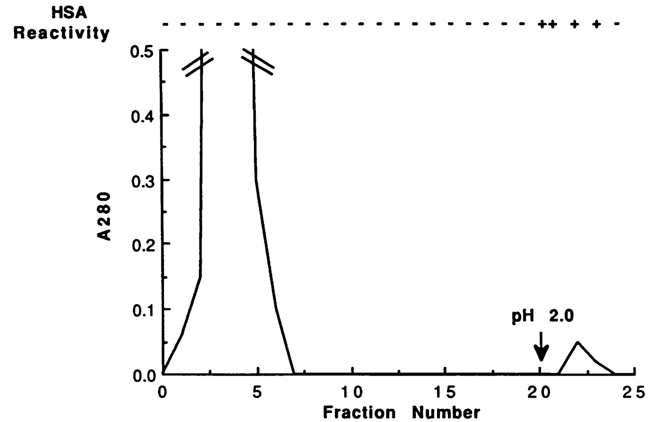

FIG. 3. Affinity purification of the albumin receptor in a neutral heat extract of bovine group G streptococcal strain BG1. Ten milliliters of a neutral heat extract of strain BG1 was applied to a column of immobilized HSA. The column was washed with phosphate-buffered saline until the optical density at 280 nm returned to baseline, and bound proteins were then eluted in a single step with 0.1 M glycine-HCl (pH 2.0). Two-milliliter fractions were collected, and 100-μl aliquots of each (neutralized, if necessary) were dot blotted onto nitrocellulose and probed for reactivity with radiolabeled HSA as described in Materials and Methods. The reactivity of each fraction is shown above the affinity column profile. The quantity of albumin-binding protein in each fraction (− to ++) was scored by measuring the diameter of the dot on the resulting autoradiograph.

to be contained in the fractions eluted from the albumin affinity column at low pH (Fig. 3). The affinity-purified proteins displayed the same binding pattern by Western blotting as shown in Fig. 1A, indicating that there was no modification of the albumin-binding proteins during purification (data not shown). This material was used as the source of immunogen to prepare polyclonal antibodies, and the affinity-purified protein could be radiolabeled with ^{125}I without loss of its ability to bind immobilized HSA (see below). The polyclonal antibody was capable of inhibiting the binding of ^{125}I-labeled HSA to the intact bacteria and in Western blotting studies was reactive with the multiple-molecular-weight forms of extracted albumin receptor observed in Fig. 1 (data not shown).

TABLE 1. Percent binding of ^{125}I-labeled HSA to bovine group G streptococci[a]

Strain[b]	% Binding with indicated no. of bacteria		
	10^9	10^8	10^7
BG1	66 ± 1	58 ± 1	27 ± 0
BG2	54 ± 5	50 ± 0	27 ± 0
BG3	59 ± 0	52 ± 1	28 ± 0
BG4	62 ± 0	54 ± 2	21 ± 0
BG5	62 ± 2	57 ± 2	27 ± 0
BG5 BB1[c]	68 ± 0	55 ± 0	17 ± 0
BG5 BB2[c]	70 ± 0	60 ± 1	26 ± 0
BG5 AB3[c]	65 ± 3	59 ± 1	27 ± 0
BG5 CB4[c]	70 ± 2	63 ± 3	36 ± 1
BG6	50 ± 1	53 ± 1	36 ± 1
BG7	55 ± 1	53 ± 0	26 ± 1
64/14	2 ± 0	0 ± 0	6 ± 0
G1400	50 ± 1	27 ± 0	12 ± 0

[a] Duplicate samples containing the stated number of bacteria were incubated with radiolabeled HSA (approximately 30,000 cpm) in a total volume of 500 μl of VBS-gel for 1 h at 37°C. The bacteria were washed twice with 2.0 ml of EDTA-gel, and the percent binding of ^{125}I-labeled HSA was determined.

[b] BG1 thru BG7 are bovine group G streptococcal isolates. 64/14 is a mouse-passaged human group A strain (36), and G1400 is a protein G-positive human group G streptococcal isolate (7).

[c] Colony-selected substrain of BG5 expressing enhanced levels of type IV IgG-binding proteins (19).

Comparison of the functional properties of the albumin receptors on human and bovine group G streptococci. In the next series of studies the functional properties of a representative human group G (G1400) and a bovine group G (BG1) streptococcus were compared in a variety of competitive inhibition assays. In the first series of experiments the species reactivity profiles of the two albumin receptors were compared. For these studies the abilities of different species of unlabeled albumin to inhibit binding of ^{125}I-labeled HSA to bovine group G streptococcal strain BG1 or to human group G streptococcal isolate G1400 were compared. The results presented in Table 2 demonstrate that both sources of group G streptococci displayed similar patterns of reactivity with HSA, and the shapes of the inhibition curves for the strains were similar. However, the albumin receptor associated with the bovine group G strain displayed a much wider range of reactivity with different species of albumin (Table 2), suggesting that the albumin-binding domain(s) of the two proteins were not identical.

The soluble albumin-binding proteins were compared for their ability to inhibit binding to immobilized HSA of either radiolabeled wild-type protein G (a molecule containing the human group G albumin receptor) or the radiolabeled, affinity-purified, albumin-binding protein isolated from the bovine group G strain in a sensitive competitive binding assay. The results presented in Fig. 4A demonstrate that unlabeled protein G or HSA could inhibit binding of the ^{125}I-labeled bovine group G albumin receptor to immobilized HSA. This cross-inhibitory potential was confirmed by using ^{125}I-labeled protein G (the human group G albumin receptor) as a tracer and comparing inhibition by unlabeled fluid-phase protein G or bovine group G albumin receptor (Fig. 4B). Soluble albumin receptor or protein G was also shown to be capable of inhibiting ^{125}I-labeled HSA binding to either the heat-killed human group G streptococcal strain G1400 or to

TABLE 2. Percent inhibition of binding of ^{125}I-labeled HSA to streptococci by different species of albumin[a]

Albumin	Strain[b]	% Inhibition of binding at albumin concn (ng)				
		1,000	333	111	37	12
Human	BG1	90 ± 1	78 ± 1	54 ± 1	27 ± 0	9 ± 1
	G1400	77 ± 0	54 ± 2	20 ± 1	9 ± 1	4 ± 1
Baboon	BG1	83 ± 0	68 ± 0	47 ± 0	30 ± 1	14 ± 0
	G1400	42 ± 0	8 ± 1	4 ± 5	0 ± 0	0 ± 0
Mouse	BG1	86 ± 0	73 ± 1	62 ± 2	33 ± 2	10 ± 0
	G1400	19 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Guinea pig	BG1	72 ± 1	53 ± 0	29 ± 4	17 ± 2	6 ± 0
	G1400	72 ± 2	44 ± 0	21 ± 3	2 ± 1	0 ± 0
Horse	BG1	42 ± 3	15 ± 2	10 ± 0	0 ± 0	0 ± 0
	G1400	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cow	BG1	42 ± 1	20 ± 0	4 ± 3	1 ± 1	1 ± 8
	G1400	9 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sheep	BG1	25 ± 0	9 ± 0	1 ± 1	0 ± 0	0 ± 0
	G1400	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Goat	BG1	17 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	G1400	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rabbit	BG1	16 ± 1	3 ± 2	0 ± 4	0 ± 0	0 ± 0
	G1400	7 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ovalbumin	BG1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	G1400	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] Duplicate samples containing 5 × 10^7 bacteria were incubated with various concentrations of each species of albumin and radiolabeled HSA (approximately 30,000 cpm) in a total volume of 500 μl of VBS-gel for 1 h at 37°C. The bacteria were washed twice with 2.0 ml of EDTA-gel, and the percent inhibition of ^{125}I-labeled HSA binding was determined by comparing the number of counts bound by the bacteria in the presence and absence of cold competition.

[b] BG1 is an albumin receptor-positive bovine group G streptococcus. G1400 is a human group G streptococcus expressing high levels of protein G.

the heat-killed bovine group G streptococcal strain BG1 (Fig. 4C).

Taken together, these results indicate that the two sources of albumin receptor were functionally very similar both in their site of reactivity with HSA and with respect to their relative binding properties. However the species of albumin bound by the two proteins demonstrated significant differences (Table 2).

Comparison of albumin receptors expressed by bovine and human group G streptococcal isolates at the DNA level. The DNA sequences coding for the albumin-binding domains of protein G from three human group G isolates have been sequenced and shown to be highly conserved (9, 11). A unique 21-base oligonucleotide from this DNA region was prepared, end labeled with [^{32}P]ATP, and used to probe chromosomal DNA from five protein G-positive human group G and five protein G-positive human group C streptococcal isolates. All 10 isolates were found to yield positive hybridization signals (Fig. 5). When similar studies were carried out with the albumin receptor-positive bovine group G streptococcal isolates, none of the eight strains tested showed significant hybridization with the specific probe (Fig. 5).

Comparison of the antigenic relatedness of the albumin receptors expressed by bovine and human group G streptococci. For studies to compare the two albumin receptor

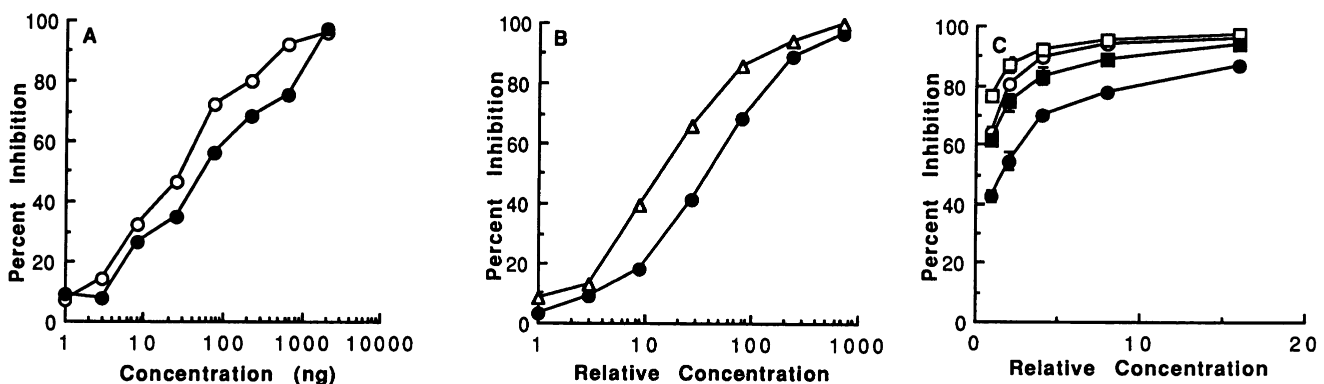

FIG. 4. Comparison of the functional activities of albumin receptors from bovine group G streptococcal strain BG1 and from protein G isolated from human group G streptococcal strain G1400 (protein G). (A) Inhibition of binding of radiolabeled bovine group G albumin receptor to immobilized HSA by HSA (●) or wild-type protein G (○). (B) Inhibition of radiolabeled wild-type protein G binding to immobilized HSA by an affinity-purified extract of the protein G-positive human group G streptococcal strain G1400 (●) or an affinity-purified extract of the bovine group G strain BG1 (△). A relative concentration of 1 is equivalent to a 1:7,290 dilution of the extract. (C) Inhibition of radiolabeled HSA binding to either bovine group G (open symbols) or human group G (solid symbols) streptococci by an affinity-purified extract of the protein G-positive human group G streptococcal strain G1400, (■ and □) or an affinity-purified extract of the bovine group G strain BG1 (● and ○). A relative concentration of 1 is equivalent to a 1:80 dilution of the extract.

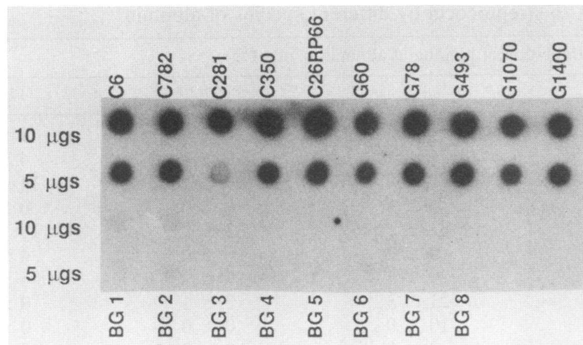

FIG. 5. Comparison of the reactivities of chromosomal DNA from various bovine group G streptococci and human group C and G strains, using an oligonucleotide specific for the albumin-binding region of protein G. Samples containing 10 or 5 µg of denatured chromosomal DNA from each strain were applied to the filter and examined for hybridization with the specific probe as described in Materials and Methods. The autoradiograph was obtained by exposing the blot to X-ray film for 48 h at −70°C.

proteins antigenically we prepared polyclonal antibodies in chickens to either the human group G streptococcal protein, protein G, or the affinity-purified albumin receptor from the bovine group G strain. The polyclonal antibody to protein G inhibited the binding of ^{125}I-labeled wild-type protein G to either immobilized HSA or immobilized human IgG. This antiserum was affinity purified on a column of immobilized recombinant protein G. The commercially available recombinant protein G preparation had been engineered to eliminate the albumin-binding domains present in the wild-type protein. Antibodies which recognized the albumin-binding domains of wild-type protein G were recovered in the unbound fractions passing directly through the column. Antibodies specific for the IgG-binding domains of protein G bound to the column and could be eluted at low pH (data not shown).

A monospecific polyclonal antibody was prepared to the affinity-purified bovine group G albumin receptor as described in Materials and Methods. The resulting antibody could inhibit the binding of ^{125}I-labeled albumin receptor to immobilized HSA (Fig. 6A), while it had a low level of inhibitory potential against protein G (Fig. 6B). Antibody from the chicken prior to immunization was without any inhibitory potential (data not shown). The antibody to each bacterial albumin-binding protein was used to compare the antigenic relatedness of the human and bovine group G streptococcal albumin receptors. The results presented in Fig. 6A demonstrate that the antibody to the albumin-binding domain of protein G had minimal inhibitory effect on the binding of ^{125}I-labeled albumin receptor to immobilized HSA. However, the antibody was efficient in inhibiting the binding of ^{125}-I labeled protein G to immobilized HSA (Fig. 6B).

DISCUSSION

Albumin receptors on the surface of a variety of streptococci isolated from humans or animals have been described (16, 30–33). Five functionally distinct albumin receptors have been classified based on their ability to bind albumin from different species (16, 30–33). The albumin-binding receptors on human (type a) and bovine (type b) group G streptococci display very similar reactivities with HSA. The type a albumin receptor was found to be expressed on type III Fc-binding-protein (protein G)-positive human group G isolates, and binding of albumin and immunoglobulin to these bacteria were found to be independent functions (4, 10, 16). Initially, this finding was taken to indicate that the bacteria expressed two independent surface binding proteins with specificity for albumin and immunoglobulin, respectively (16). Subsequent studies by Björck and colleagues indicated that a single bacterial protein molecule, protein G, isolated from human group G streptococci could account for both activities (2, 4, 28). This conclusion was verified when the recombinant protein was prepared and albumin-binding and IgG-binding regions could be subcloned and expressed independently (27). In studies with isolated protein G it has been demonstrated that the ability of wild-type protein G to bind immunoglobulin is not altered in the presence of a large molar excess of albumin and vice versa (10).

The reported findings that group G strains isolated from

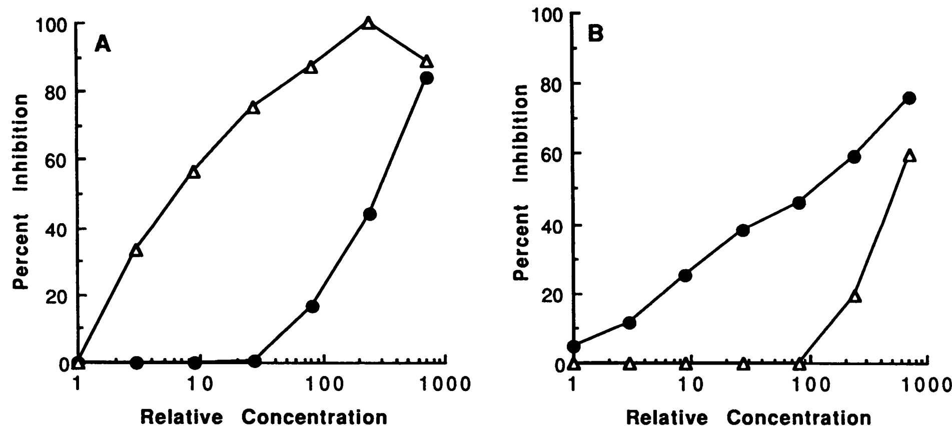

FIG. 6. Inhibition of binding of ^{125}I-radiolabeled albumin receptors from human and bovine group G streptococci to immobilized HSA by polyclonal antibodies. (A) Inhibition of ^{125}I-labeled albumin receptor binding to immobilized HSA by anti-albumin receptor antibody (△) or by antibody to the albumin-binding region of protein G (●). (B) Inhibition of ^{125}I-labeled wild-type protein G binding to immobilized HSA by anti-albumin receptor antibody (△) or by antibody to the albumin-binding region of protein G (●). All estimates were carried out in duplicate, and less than 5% variance was observed between samples. Inhibition of binding by normal chicken serum was less than 5% in each assay. A relative concentration of 1 corresponds to a 1:21,870 dilution of the antiserum.

bovine and human sources express albumin receptors which differ in their quantitative reactivity with horse, bovine, and goat albumin but are otherwise indistinguishable (31, 33) prompted us to undertake a detailed comparison of the albumin-binding activities. Of particular interest was the finding that in our collection of bovine group G isolates, 100% of the strains displayed albumin-binding activity while less than 20% displayed immunoglobulin-binding activity (19). In light of the combined expression of albumin-binding and immunoglobulin-binding activities on a single protein in the human strains we wanted to test the hypothesis that albumin binding represented the most primitive form of reactivity with mammalian serum proteins and that immunoglobulin-binding activity would represent a higher level of survival advantage to the bacteria as it evolved against the selective pressures of more advanced mammalian host defense mechanisms.

The studies presented in this paper demonstrate that all of the bovine group G strains studied express an albumin receptor, the predominant molecular form being an $\sim$45,000-M_r protein. Lower-molecular-weight albumin-reactive bands were also observed in heat extracts and most probably represented breakdown products, since antibody to the $\sim$45,000-M_r protein recognized all albumin-binding forms. The major albumin-binding protein purified in this study was larger than the 30,000-dalton protein obtained by Widebäck and Kronvall following hot acid extraction of a bovine group G strain in their collection (32). The albumin-binding activity was shown to be distinct from the type IV IgG-binding activity (compare Fig. 1A and B), and there was no evidence in any bovine group G strain studied that a single molecule displayed both IgG- and albumin-binding activity. Monospecific polyclonal antibodies to the isolated bovine group G albumin-binding protein (type b) were raised in chickens and displayed a low level of cross-reactivity with protein G (type a). A similar antibody to the albumin-binding domain of protein G (type a) inhibited efficiently the functional activity of the type a receptor and also displayed a low level of activity against the type b receptor. However, dot blot analysis of chromosomal DNA from human and bovine group G strains probed with a specific DNA probe for the type a (human group G) albumin-binding domain demonstrated positive hybridization with five protein G-positive human group G and five protein G-positive human group C streptococcal isolates; however, none of the bovine group G strains displayed any reactivity with the specific oligonucleotide probe.

The observation that albumin receptors are expressed by the vast majority of bovine and human group G isolates suggests that this property has provided the bacteria with some survival advantage. The finding that in the human isolates, but not in the bovine isolates, the same molecule expresses this albumin-binding activity along with an immunoglobulin-binding activity is intriguing. Albumin and immunoglobulin G represent the most abundant mammalian serum proteins and potentially a bacterium expressing these activities could be "camouflaged" against host defense mechanisms by coating itself with host proteins, enabling the bacterium to appear as self. While this mechanism might provide the human group G strain with a survival advantage in humans, it is difficult to make a similar argument for the bovine group G isolate. These strains bind bovine serum albumin with a much lower affinity than they display towards HSA (Table 2), and when they do express immunoglobulin-binding proteins they display their greatest reactivity towards rabbit immunoglobulins, without detectable reactivity with bovine immunoglobulins (17, 19, 24). At this time, there is no clear explanation why these different bacteria have evolved and maintained a selective albumin receptor or why these distinct receptors expressed on human and bovine group G streptococci display such similar reactivities with HSA.

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