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Receptor in Group C and G Streptococci Detects Albumin Structures Present in Mammalian Species

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The presence of albumin structures with the capacity to bind to a surface receptor in group C and G streptococci was studied in serum samples from 45 mammalian species representing 15 different orders, using an inhibition assay. The ability of animal sera to inhibit the uptake of radiolabeled human serum albumin by the streptococci indicated the presence of such albumin structures. Positive reactions were found in species of most orders tested, with *Marsupialia* as a notable exception. All *Carnivora* sera tested were strongly positive. In some orders such as *Artiodactyla* both positive and negative species were identified. Serum samples from 62 bird species representing 15 orders and from 5 fish species were also tested in the inhibition assay. None of these serum samples was capable of inhibiting the uptake of human serum albumin by streptococci. Some differences were also noted in the results obtained with group C and G streptococci from human and bovine sources, respectively, indicating the presence of two types of receptors. The present studies suggest a phylogenetic origin of albumin structures with affinity for the streptococcal receptor to a period after the divergence of *Marsupialia* from the other mammalian orders.

Streptococci are capable of highly specific interactions with human proteins. These interactions are mediated by binding structures on the bacterial surface. Five different types of reactivities are known. Streptococci can bind immunoglobulin G (16, 29–31), fibrinogen (8, 19, 39), aggregated beta-2-microglobulin (17, 18), haptoglobin (23), and albumin (22, 32). Only group C and G streptococci carry surface structures with specificity for serum albumin (32). This albumin receptor is different from the other four human protein receptors, as judged by inhibition experiments and lack of correlation in individual strains. The albumin binding capacity of a group G streptococcus, G-148, is of the same order as the immunoglobulin G binding capacity of *Staphylococcus aureus* strain Cowan I (22, 32). In the present investigations we have studied in various animal species the occurrence of albumin structures with the capacity to bind to the surface receptor in group C and G streptococci. Studies of a similar kind, using protein A, have been performed of the phylogeny of immunoglobulin structures in birds and mammals (20, 21). The results of our studies of albumin-binding structures show that albumin from many mammalian species but not from bird species can bind to receptors on group C and G streptococci. Albumin-binding streptococci might therefore be used for convienient absorption of not only human serum albumin but also albumin from many other mammalian species.

MATERIALS AND METHODS

Bacterial strains. A total of 87 streptococcal strains were studied. 54 from Lancefield group C (22 *Streptococcus equisimilis*, 12 *Streptococcus zooepidemicus*, 16 *Streptococcus dysgalactiae*, and 4 *Streptococcus equii*) and 33 beta-hemolytic strains from Lancefield group G. Sixteen of the group G streptococci were isolated from clinical specimens of human origin, and there were 17 isolates from bovine milk samples. *S. zooepidemicus*, *S. dysgalactiae*, and *S. equii* will be referred to as bovine group C streptococci. Some *S. zooepidemicus* and *S. equii* strains were of horse origin. Lancefield grouping was performed by using the coagglutination method (5). A *Staphylococcus aureus* strain, Cowan I (NCTC 8530), was used as a negative control in binding assays.

Animal sera. Blood samples were obtained from birds caught at ornithological field stations in Sweden and from birds and mammals in various zoological gardens in Denmark and the United States. Blood samples from fish were kindly provided by an angler in Sweden.

Radiolabeling of albumin preparations. Human serum albumin (lot 001693; AB Kabi, Stockholm, Sweden) and essentially fatty acid-free human albumin (lot A-1887), dog albumin (lot A-4510), horse albumin (lot A-9888), and rabbit albumin (lot A-0639) (all from Sigma Chemical Co., St. Louis, Mo.) were labeled with ^{125}I (code IMS 30; Radiochemical Centre, Amersham, England), using the chloramine-T method (14,

26). Albumin, 100 μl (5 mg/ml in 0.1 M phosphate buffer, pH 7.25), was mixed with 0.2 mCi of carrier-free ^{125}I in a beaker cooled in an ice bath. After addition of 20 μl of chloramine-T (2 mg/ml), the reaction was allowed to proceed for 1 min under stirring. The labeling process was stopped by addition of 100 μl of sodium metabisulfite (5 mg/ml). Free, unreacted isotope was removed by gel filtration on a Sephadex G-25 column (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). To prevent nonspecific adsorption of labeled albumin, Tween 20 was added to all subsequent buffers at a final concentration of 0.05%.

Albumin binding assay. Overnight broth cultures of bacterial strains were washed twice and suspended in phosphate-buffered saline containing 0.02% sodium azide and 0.05% Tween 20 to 10^9 bacteria per ml, as calculated from optical density values at 620 nm on diluted samples in a Beckman CP-1 colorimeter. Binding studies were performed at room temperature in disposable polystyrene test tubes (12 by 70 mm; AB Cerbo, Trollhättan, Sweden) by adding 2×10^8 bacteria to 0.1 μg of labeled albumin. After 1 h at room temperature, 2 ml of phosphate-buffered saline containing 0.02% sodium azide and 0.05% Tween 20 was added to each tube. The bacteria were deposited by centrifugation, and the radioactivity in the pellet was measured in a gamma counter (LKB Rack Gamma 1270; Biotec, Stockholm, Sweden).

Inhibition assay. A 20-μl portion of serum or pure albumin was mixed with 0.1 μg of labeled albumin. Bacteria, 2×10^8, were added to each tube. The samples were then processed as described above for the albumin binding study. Inhibition values were expressed as percentage of maximum achievable inhibition of the uptake of each individual albumin preparation by each streptococcal strain. Inhibition values are therefore comparable. Inhibition values below 15% were regarded as negative.

RESULTS

Inhibition of albumin binding by mammalian serum samples. Serum samples from 45 mammalian species belonging to 15 orders were tested in an inhibition assay for ability to inhibit the binding of radiolabeled human serum albumin to the albumin receptor on group G streptococci. In this test system both regular, purified human serum albumin and a preparation of human serum albumin made essentially fatty acid-free were used, as well as two streptococci, one human group G (G-148) and one bovine group G (DG-6). The results of inhibition assays of mammalian sera showed varying degrees of inhibition from negative to strongly positive (Fig. 1). To permit comparisons between tests, using strains of varying degrees of albumin reactivity, the observed inhibition values were expressed as percentage of maximum achievable inhibition. One order, *Marsupialia*, with serum samples from seven different species was negative in all four test systems. Tests with serum samples from several individual rabbits showed little or no inhibition. Of all the other orders studied, there was positive inhibition in all or in some species (Table 1). *Carnivores* gave very strong inhibition in all four test systems. *Artiodactyla* represented a heterogeneous group, with both strongly positive and negative species. When essentially fatty acid-free albumin was used, the overall inhibition reactions were more pronounced, but there were no changes in the overall pattern of the results.

Comparison of human and bovine group G streptococci. When human and bovine streptococcal strains G-148 and DG-6 were compared, there were some notable quantitative and qualitative differences. The human strain was capable of binding 50% of added human serum albumin, whereas the bovine strain could bind as much as 70% of added albumin. There were also differences in the inhibition assay (Table 1). The orders *Edentata* and *Rodentia* gave only a small but positive inhibition with the human streptococcus but a very strong inhibition when the bovine streptococcal strain was used. Serum from hyrax of the order *Hyracoidea* showed the opposite pattern: good inhibition of binding of human albumin to the albumin receptors on the human streptococcus but no inhibition at all with the bovine streptococcus. *Carnivores* species tested gave a very strong inhibition in both albumin binding systems. Although *Artiodactyla* comprised a very heterogeneous order, with some high and some low inhibition values in

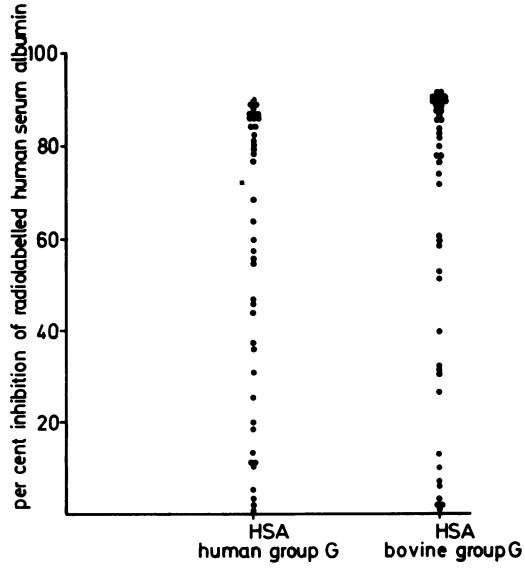

FIG. 1. Inhibitory effect of 45 mammalian serum samples on uptake of radiolabeled human serum albumin (HSA) by a human and a bovine group G streptococcus.

TABLE 1. Inhibition of human serum albumin binding to group G streptococci by various animal serum samples (45 species)

Order	No. of species	% Inhibition (range)[a]			
		Human group G *streptococcus* (G-148)		Bovine group G *streptococcus* (DG-6)	
		r-HSA	ff-HSA	r-HSA	ff-HSA
Marsupialia	7	8 (4–15)	7 (3–14)	6 (1–13)	9 (0–14)
Primates	4	73 (40–90)	73 (31–90)	78 (57–88)	86 (74–91)
Edentata	3	22 (14–35)	36 (26–46)	81 (80–81)	84 (80–88)
Pholidota	1	85	88	86	78
Lagomorpha	1	14	17	5	6
Rodentia	3	24 (14–43)	15 (11–21)	67 (36–85)	63 (32–79)
Cetacea	1	73	84	72	90
Carnivora	12	84 (77–90)	72 (59–78)	88 (81–91)	76 (59–83)
Pinnipedia	2	50 (29–72)	50 (20–81)	75 (71–80)	86 (82–89)
Tubulidentata	1	22	18	57	53
Proboscidea	1	39	69	62	59
Hyracoidea	1	62	55	1	1
Sirenia	1	72	84	74	84
Perissodactyla	4	21 (3–59)	38 (6–56)	44 (35–51)	46 (31–61)
Artiodactyla	9	21 (0–54)	26 (0–64)	25 (2–74)	25 (2–73)

[a] Expressed as percentage of maximum achievable inhibition. r-HSA, Regular pure human serum albumin; ff-HSA, essentially fatty acid-free human serum albumin.

both systems, the levels were consistent in both systems.

Comparison of group C and G streptococci. To find out whether the differences noted reflected general differences between all human and bovine group G streptococci, another series of experiments was performed. The inhibiting capacity of human, armadillo (order *Edentata*), and hyrax (order *Hyracoidea*) serum samples on binding of radiolabeled human serum albumin to different human and bovine group G streptococci was tested (Fig. 2). The inhibition assay was also performed with human and bovine group C streptococci since group C and G streptococci are very closely related. Of 87 group C and G streptococci tested, 56 were capable of binding human serum albumin. Only 5 of 32 bovine group C streptococci could bind human serum albumin. These five were all *S. dysgalactiae* strains. Twelve *S. zooepidemicus* and four *S. equii* strains did not bind human serum albumin. When bovine streptococci were used, the armadillo serum inhibited the uptake of labeled human serum albumin almost as much as unlabeled human albumin did, whereas hyrax serum had no effect. Human group C and G streptococci and bovine group C and G streptococci behaved as uniform populations regarding the inhibition patterns. This indicated that there are two slightly different types of receptors for human albumin on streptococci, one on bovine and one on human group C and G streptococci.

Inhibition of albumin binding by mammalian albumin preparations. To verify that the albumin component of the serum samples was responsible for inhibition of the binding of radiolabeled human serum albumin to the receptors on the streptococci, purified albumin from seven different animals was tested in an inhibition assay. Figure 3A shows the effect of animal albumin preparations competing with 0.1 μg of radiolabeled human serum albumin for the receptors on a human group G streptococcal strain (G-148). Five concentrations, from 8 to 800 μg, of each animal albumin preparation were tested. Dog, human, and guinea pig albumin had very strong inhibiting effects on the uptake of labeled human serum albumin. Horse albumin inhibited the

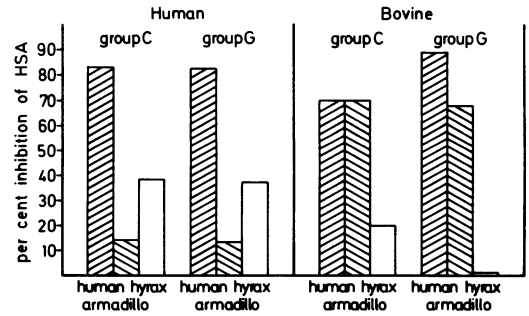

FIG. 2. Inhibition by human, armadillo, and hyrax sera of the uptake of radiolabeled human serum albumin (HSA) by 22 human and 5 bovine group C and 16 human and 17 bovine group G streptococcal strains.

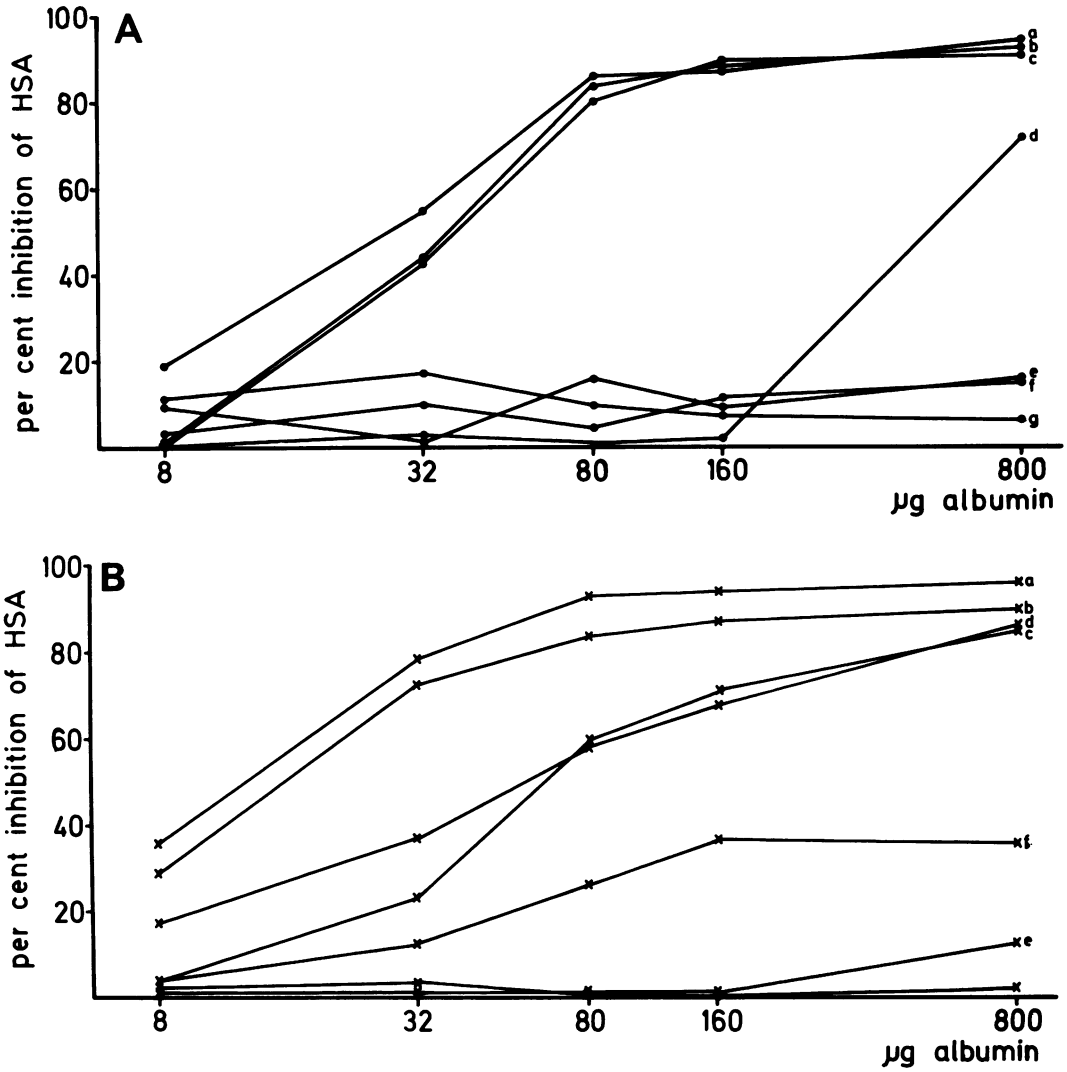

FIG. 3. Inhibition experiments with increasing amounts of dog (a), human (b), guinea pig (c), horse (d), bovine (e), rabbit (f), and goat (g) albumin preparations competing with 0.1 μg of human serum albumin (HSA) for binding to (A) a human group G streptococcal strain (G-148) and (B) a bovine group G streptococcal strain (DG-6).

uptake of human serum albumin only in the highest concentration, whereas bovine, rabbit, and goat albumin had no inhibitory effect.

When a bovine group G streptococcal strain was used, there was another pattern of inhibition (Fig. 3B). The albumin preparations could be divided into four groups. Dog and human albumin showed a very good inhibitory effect, as in the human streptococcus system. Horse and guinea pig albumin had a moderately inhibitory effect. Goat albumin had only a slight effect, and bovine and rabbit albumin did not inhibit the uptake of radiolabeled human serum albumin by the bovine streptococcus.

Uptake of mammalian albumin preparations to streptococci. In the investigations described above, inhibition of the uptake of radiolabeled human serum albumin by albumin receptors on streptococci was studied. To get more direct information on the binding to these receptors, the direct uptake of some radiolabeled mammalian albumin preparations was also measured. The uptake of radiolabeled dog, horse, rabbit, and human albumin by seven human and four bovine group C streptococci and five human and seven bovine group G streptococci is illustrated in Fig. 4. Dog albumin showed a very good binding to C and G streptococci. Its binding to bovine group

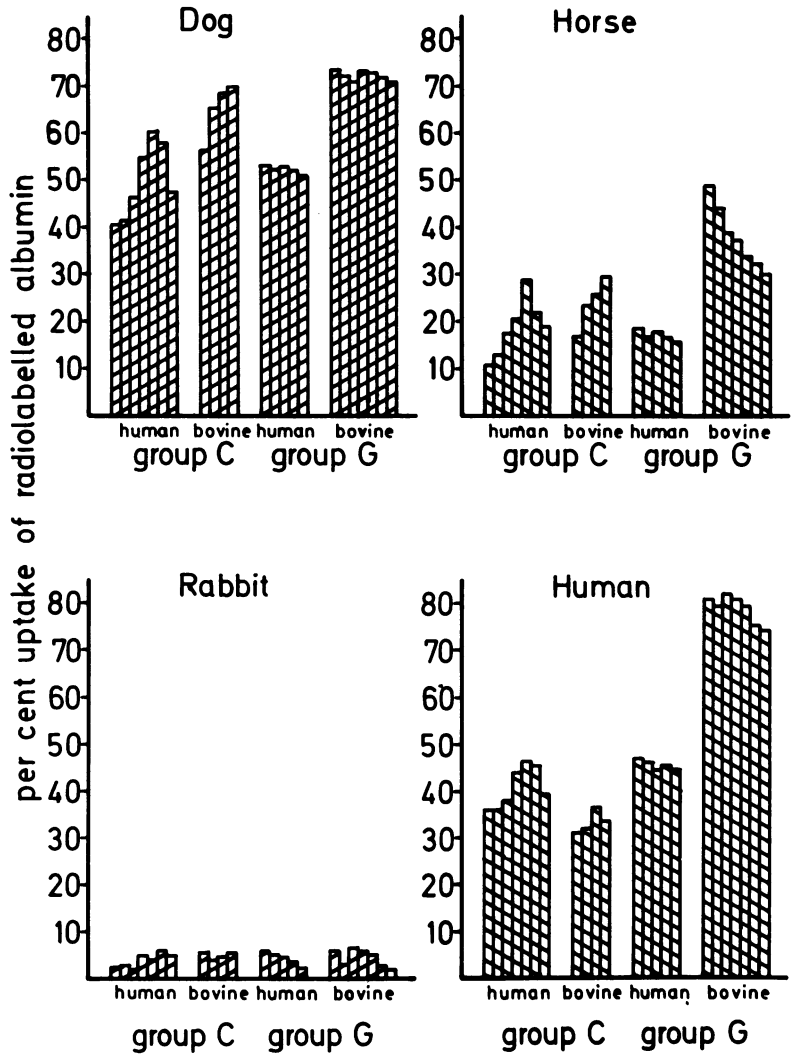

FIG. 4. Binding of dog, horse, rabbit, and human albumin preparations to seven human and four bovine group C streptococci and five human and seven bovine group G streptococci.

C streptococci was even stronger than the binding of human albumin to group C streptococci. Horse albumin showed a lower binding activity, the highest being to bovine group G streptococci. Rabbit albumin did not bind to albumin receptors on group C and G streptococci.

Inhibition of albumin uptake by nonmammalian animal sera. Serum samples from 62 bird species from 22 families among 15 orders were tested for albumin binding to the albumin receptors in an inhibition assay. None of the serum samples was capable of inhibiting the uptake of radiolabeled human serum albumin to the albumin receptors on human and bovine group G streptococci. All species were therefore completely negative in these tests. Serum samples from five fish species were also tested, with the same negative results.

DISCUSSION

Streptococci comprise a large and biologically diverse group of microorganisms. Several characteristics are common to group A, C, and G streptococci. They invade the same areas in the human body and can cause severe and even fatal infections (3, 6, 7, 9, 13, 33–35). There are several factors separating group A strains from group C and G streptococci. M-proteins are limited to A strains, with rare exceptions (11, 25). Group A streptococci are found mostly in humans, whereas group C and G strains are

common causative agents also in animal infections (4, 10, 12, 15, 27, 28). Similarly, only group C and G streptococci carry surface receptors with specificity for serum albumin (22, 32). This albumin binding cannot be mediated by lipoteichoic acid for several reasons. Lipoteichoic acid is a regular component with a uniform structure found in most gram-positive bacteria, including streptococci. The restriction of albumin reactivity to group C and G streptococci is difficult to reconcile with this fact. The equilibrium between albumin and albumin receptors on streptococci is approached in less than 20 min, whereas it takes 12 h for a similar degree of lipoteichoic acid-albumin binding (36, 37). In our experiments we also included a detergent, Tween 20, which is capable of eluting lipoteichoic acid from albumin.

In the present investigations the inhibition of albumin uptake by mammalian serum samples was studied. Inhibition results correlate well within the mammalian orders in each of the two assays, using a human (G-148) and a bovine (DG-6) group G streptococcus, respectively (Table 1). All seven *Marsupialia* serum samples were negative (3 to 14 and 1 to 13%, respectively) in the inhibition assays, using regular, pure serum albumin and the two streptococcal strains. The negative results of all seven samples from the order *Marsupialia* differ highly significantly ($P < 0.0005$) from the results with all other mammalian serum samples. From each of seven different orders, serum samples from only one species were available. Six of these orders were positive. All serum samples tested from rabbits (order *Lagomorpha*) were negative. This result does not, however, differ statistically significantly from the other mammalian orders. All 12 *Carnivora* serum samples showed very strong positive inhibition in both streptococcal systems. The differences in inhibition results when regular pure human human serum albumin and essentially fatty acid-free human serum albumin were used were negligible. Bird and fish serum samples included in the studies gave no inhibition of human serum albumin uptake by human or bovine streptococci. Fish serum, however, does not contain regular serum albumin (U. Sörensen, personal communications). Birds, on the other hand, produce regular serum albumin, but all 62 species tested were negative.

Since bird and fish serum samples could not inhibit the binding of human serum albumin to streptococci, the origin of the albumin structures identified by the streptococcal receptor can be dated to the mammals' estimated last common ancestor living about 285 million years ago. In our experiments serum samples from seven different species in the mammalian order *Marsupialia* were negative in inhibition assays, indicating lack of streptococcal reactive albumin structures. This difference was statistically significant ($P < 0.0005$) All of the other mammalian orders exept *Lagomorpha* had at least some positive serum samples. The negative results with rabbit serum were not statistically significant. Analysis of amino acid sequences of myoglobulin and hemoglobin has provided a date for the kangaroo-eutherian divergence, about 138 million years ago (1). It follows that the albumin structures studied can be dated to later than this age.

The present investigations indicate that there are two different types of albumin receptors, one on human strains and one on bovine strains. There are, however, no differences in albumin receptors on human group C and G streptococci or on bovine group C and G strains. We have studied 87 strains of group C and G streptococci: 22 *Streptococcus equisimilis*, 12 *S. zooepidemicus*, 16 *S. dysgalactiae*, 4 *S. equii*, and 16 human and 17 bovine group G streptococcal strains. The albumin receptor is present in *S. equisimilis* (human group C), *S. dysgalactiae* (bovine group C), and in human and bovine group G streptococci. The inhibition results show that the albumin receptor in *S. equisimilis* and human group G streptococci is different from that in *S. dysgalactiae* and bovine group G streptococci (Table 1). This indicates that human strains of group G streptococci are more closely related to human group C strains than to bovine group G streptococci, and bovine strains of group G streptococci are more closely related to bovine group C strains than to human group G streptococci. All of the investigated group G strains were beta-hemolytic; alpha-hemolytic group G strains do not have albumin receptors (32). *S. dysgalactiae* is an alpha-hemolytic group C strain.

The serum protein binding studied is of interest from a biological point of view considering that albumin is the most abundant blood protein. The albumin receptors on streptococci are of high avidity, with an affinity in the order of 10^7 liters/mol. The average bacterial cell carries more than 80,000 binding sites (32). Pathogenic bacteria often show several lines of host interaction capabilities. For human group C and G streptococci, albumin binding might be one of them.

Staphylococci carrying protein A have been used in laboratory work for selective absorption of immunoglobulins (2, 24, 38). Albumin-binding streptococci can serve similar purposes (22). Bacteria can also be immobilized and entrapped in polyacrylamide gel, with most of the albumin binding sites free (24a). This possibility of using bacterial particles for adsorption purposes on a preparative scale might be of value in various laboratory procedures.

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