PHYSIOLOGY AND MANAGEMENT

Bovine Lactoferrin Receptors in *Staphylococcus aureus* Isolated from Bovine Mastitis

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ABSTRACT

A total of 103 *Staphylococcus aureus* strains isolated from bovine mastitis were tested for bovine lactoferrin binding in a ^{125}I-labeled protein binding assay. More than 85% of the strains demonstrated high to moderate and a few showed little or no binding. Bovine lactoferrin binding to *S. aureus* cells was high when grown on blood, nutrient, or proteose-peptone agar, but the binding capacity was low with cells grown on salt rich media, in skim milk, or in broth. The kinetics of ^{125}I-labeled bovine lactoferrin binding required approximately 90 min for complete saturation with optimal interaction in the pH range 4.0 to 7.0. The lactoferrin-staphylococci interaction was specific with a high affinity (association constant, K_a 14×10^6 L/mol). Scatchard plot analysis estimated the number of binding sites per cell at 7200 on strain SA-340. Unlabeled bovine lactoferrin effectively displaced the binding of the labeled ligand to strain SA-340 in a dose-dependent manner. Bovine lactoferrin binding was inhibited or displaced by human lactoferrin. Various plasma, connective tissue, or mucosal secretory proteins tested did not inhibit lactoferrin-staphylococci interaction. Bovine lactoferrin binding components on SA-340 were resistant to glycolytic enzymes and moderately susceptible to proteolytic digestion. Two proteins with an estimated molecular weight of approximately 92 and 67 kDa were identified as bovine lactoferrin binding components of *S. aureus* strain SA-340.
(**Key words**: lactoferrin, binding, *Staphylococcus aureus*)

Abbreviation key: **bLf** = bovine lactoferrin, **hLf** = human lactoferrin, **HRPO** = horseradish peroxidase, **Lf** = lactoferrin, **PBS** = phosphate-buffered saline, **PMN** = polymorphonuclear leukocytes.

INTRODUCTION

Bovine lactoferrin (bLf) is a 92.1-kDa, acute phase, iron-binding protein occurring in milk, various biological secretions, and polymorphonuclear leukocytes (PMN) (11, 28). Bovine lactoferrin concentrations vary in the range of .1 to .3, 2 to 5, and 20 to 30 mg/ml in normal milk, colostrum, and during glandular involution, respectively, depending on the physiologic status of the bovine mammary gland (21, 29). During acute bovine mastitis, bLf concentration in the lacteal secretions increase by 30-fold, corresponding to the severity of infection (7).

The biological role of lactoferrin (Lf) includes the amplification of the inflammatory response by promoting the adhesion and aggregation of PMN to the endothelial surface. Lactoferrin stimulates the phagocytic and cytotoxic property of macrophages and regulates myelopoiesis by causing a feedback inhibition of

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granulocyte-monocyte colony-stimulating factor (3). Specific binding of iron-saturated human lactoferrin (hLf) to the membrane of mouse peritoneal macrophages has been described (26). A mannose-sensitive, calcium-dependent, specific binding of hLf to the human adherent mononuclear cells has also been reported (2).

As a major component of the specific granules of PMN, Lf contributes to both hydrogen peroxide-dependent and peroxide-independent bacterial killing. The iron-binding property enables Lf to limit the iron availability to bacteria and ultimately causes bacteriostasis (1). Lactoferrin enacts cooperatively with IgA from human milk or IgG_1 from bovine colostrum, resulting in a more effective bacteriostasis (22).

Bovine milk, although rich in essential nutrients, is bacteriostatic in vivo due to the presence of immunoglobulins, phagocytic cells, complement components, lysozyme, lactoperoxidase, and the Lf system (18). During an inflammatory response, leukocytes consisting mainly of PMN infiltrate the bovine mammary gland and provide a primary host defense (6). The PMN release bLf and increase bLf during bovine mastitis. Thus, the bacteriostatic property of bLF is an important factor in the prevention of udder infections (7). However, the interaction of bLf with *Staphylococcus aureus* strains associated with infections of the bovine mammary gland is not known. The purpose of this study was to determine the specific binding of bLf to *S. aureus* strains associated with bovine mastitis.

MATERIALS AND METHODS

Bacteria

A total of 103 *S. aureus* strains isolated from chronic and acute bovine mastitis were selected for the bLf binding study. Bacterial strains were stored in glycerol at −80°C, subcultured, and subsequently cultivated on blood agar base number 2 (Oxoid Limited, Basingstoke, England) supplemented with 10% human blood at 37°C for 18 h. The effect of culture media on bLf binding was studied by growing *S. aureus* strain SA-340 on nutrient agar, on staphylococcus medium 110, and in nutrient broth (Oxoid Limited); on proteose-peptone, mannitol salt, brain-heart infusion agars, and in brain-heart infusion broth (Difco Laboratories, Detroit, MI); and in skim milk (Swedish Dairies Association, Malmö, Sweden). For ^{125}I-labeled bLf binding experiments, bacteria were grown at 37°C for 18 h, harvested, washed, and resuspended in phosphate-buffered saline (PBS), pH 7.2. Bacterial suspension was adjusted to a density of ~10^{10} cells/ml optically at 540 nm absorbance, and cells were also counted under a phase-contrast microscope using a modified Burker chamber (depth, .01 mm). All further experiments were performed with blood agar grown bacterial cells unless otherwise stated.

Chemicals

Bovine lactoferrin (purified from bovine milk) was kindly provided by H. Burling, Swedish Dairies Association, Malmö, Sweden, and was used throughout the study. Bovine lactoferrin (from bovine colostrum) and hLf (from human milk) were purchased from US Biochemicals Corp. (Cleveland, OH). Bovine lactoferrin protein preparations obtained from the above two different sources demonstrated similar binding properties. Fibronectin was purified from bovine plasma, which was provided by Janos Erdei. Bovine transferrin, bovine fibrinogen, bovine serum albumin, bovine IgG, ovalbumin, trypsin, pepsin, proteinase K, neuraminidase, β-D-galactosidase, α-1-fucosidase, deoxyribonuclease II (from bovine spleen; EC.3.1.21.1), ribonuclease A (from bovine pancreas; EC.3.1.27.5), lysostaphin, N-acetyl-neuraminic acid, N-acetylgalactosamine, N-acetylglucosamine, *p*-nitrophenyl-β-D-glucoside, *o*-nitrophenyl-β-D-galactoside were purchased from Sigma Chemicals Co. (St. Louis, MO). Chemicals used for the preparation of buffers were of analytical grade.

Binding Assay

Bovine lactoferrin was labeled according to a modified chloramine T method with Na [^{125}I] (specific activity 629 GBq/mg) (DuPont Scandinavia AB, Stockholm, Sweden) using Iodobeads (Pierce Chemicals Co., Rockford, IL). Binding assays were performed as described earlier by Naidu et al. (13, 14). Briefly, 10^9 bacterial cells in a volume of .1 ml of PBS were mixed with .1 ml of ^{125}I-labeled bLf (radioactivity adjusted to 2 to 2.5×10^4 cpm,

i.e., approximately 8 ng, when diluted in cold PBS). After 1 h incubation at room temperature, 2 ml of ice-cold PBS containing .1% Tween 20 were added to the tubes. The suspension was centrifuged at 4500 × *g* for 15 min, and the supernatant was aspirated. Radioactivity retained in the bacterial pellet was measured in a gamma counter (LKB Wallac Clingamma 1271, Turku, Finland). Residual radioactivity from incubation mixtures containing no bacteria, i.e., background, was 2.5%, whereas the nonspecific ^{125}I-labeled bLf binding in the presence of excess of unlabeled bLf was approximately 7.5% of the total labeled protein added. Thus a binding value of below 10% of the total ^{125}I-labeled bLf added was considered as negative. Samples were tested in triplicate, and each experiment was repeated at least two times unless otherwise stated.

Displacement and Competitive Inhibition of Binding

For the determination of the binding specificity, increasing amounts of unlabeled bLf in .1 ml PBS were mixed with equal volume PBS containing ~8 ng of ^{125}I-labeled bLf. Approximately 10^9 bacteria were added to this solution (final volume .3 ml), and the mixture was incubated at room temperature for 1 h. For Scatchard analysis, increasing amounts (.1 to 10 μg) of labeled bLf (with a low specific activity) were allowed to interact with 10^9 bacteria, either in the absence (total binding) or in the presence (nonspecific binding) of 50-fold excess of unlabeled bLf.

The competitive inhibitory effect of different proteins, such as transferrin, hemin, IgG, fibronectin, fibrinogen, fetuin, and of different carbohydrates such as glucose, mannose, sorbitol, ribose, fucose, galactose, *p*-nitrophenyl-β-D-glucoside, *o*-nitrophenyl-β-D-galactoside, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, and N-acetylneuraminic acid on ^{125}I-labeled bLf binding to SA-340 was tested. A volume of .1 ml of .1% protein or carbohydrate solution was mixed with approximately 10^9 bacteria (in .1 ml volume) and kept at room temperature for 30 min. Finally, .1 ml (~8 ng) of ^{125}I-labeled bLf was added, and the mixture was incubated at room temperature for 1 h. The ^{125}I-labeled bLf binding measurements were made as described.

Treatment of SA-340 with Enzymes

Bacteria (.1 ml containing 10^9 cells) were treated with proteases or glycosidases (in .1 ml volume) at room temperature. Trypsin (25,000 units/ml) hydrolysis was performed in .15 *M* PBS, pH 7.4, and the reaction was blocked with soybean trypsin inhibitor. Pepsin (10,000 units/ml) treatment was performed in 100 m*M* sodium acetate buffer, pH 4.5, and the hydrolysis was stopped by increasing the pH of the reaction mixture to 7.4. Proteinase K (100 units/ml) digestion was carried out in 40 m*M* potassium phosphate buffer, pH 7.5, and the reaction was inhibited by addition of .5 *M* phenylmethylsulfonylfluoride. α-1-Fucosidase (.5 units/ml) and β-D-galactosidase (1000 units/ml) treatments were performed in .17 *M* PBS, pH 7.4. Finally, neuraminidase (10 units/ml) treatment was done in .1 *M* citrate buffer, pH 5.0. After 1 h of incubation, bacteria were washed thoroughly with PBS. During the treatment, bacterial sedimentation was prevented by gentle rotation. Bacteria were washed carefully and resuspended in .1 ml of PBS, and the ^{125}I-labeled bLf binding assay was performed as described. Untreated cells suspended in appropriate buffers served as controls during the calculation of percentage susceptibility values.

Heat and Periodate Treatment of SA-340

One milliliter of bacteria (10^{10} cells/ml) was heated at 50 and 80°C for 1 h, with gentle shaking in a water bath. Furthermore, 1 ml of bacterial suspension (10^{11} cells) was mixed with 5 mg of sodium-*p*-periodate (lot 58F.0310; Sigma). The mixture was protected from light and kept in cold room with gentle agitation. After 24 h incubation, the mixture was centrifuged at 500 rpm for 5 min. Bacterial cells in the supernatant were aspirated and dialyzed against PBS for 24 h. Finally, the density of the sodium periodate-free cell suspension was adjusted to 10^{10} cells/ml. Cells obtained from the above two treatments were tested for ^{125}I-labeled bLf binding as described.

Extraction of *Staphylococcus aureus* Cell Surface Components

Staphylococcus aureus strain SA-340 cell surface components were extracted according to Rydén et al. (19). In brief, 20 g wet weight of

bacteria were suspended in 200 ml of 50 m*M* Tris-HCl, pH 7.5 (Tris-buffer), containing .145 *M* NaCl, 2 mg of lysostaphin, 2 mg of DNase, and 2 mg of RNase. After incubation with constant shaking (150 rpm) at 37°C for 2 h, the reaction mixture was centrifuged (4500 × *g* for 30 min), and the supernatant (hereby termed "lysate") was collected. The presence of functionally active bLf binding components in the preparation was detected by adding the lysate (in increasing amounts) to SA-340 whole cells and performing a ^{125}I-labeled bLf binding inhibition assay.

Furthermore, the lysate was subjected to ammonium sulfate (60%) precipitation. The precipitate was separated by centrifugation at 4000 × *g* for 30 min and redissolved in PBS. Both the precipitate and the supernatant were then dialyzed against PBS to remove ammonium sulfate and tested for the presence of bLf binding components by electrophoretic immunoblotting using horseradish peroxidase (HRPO)-labeled bLf.

Gel Electrophoresis

The SDS-PAGE was performed according to Laemmli (9). The running gel consisted of a linear 5 to 15% acrylamide gradient. Samples of a mixture of reference proteins (4 μg of each protein; Pharmacia Fine Chemicals, Uppsala, Sweden) were applied to each lane after boiling in the presence of SDS containing mercaptoethanol. After electrophoretic run, proteins were transferred to immobilon membrane (Millipore Co., Bedford, MA) by electrophoretic transfer (25). The free hydrophobic sites on the membrane were blocked by washing with Tris-buffered saline, pH 7.4, containing 1.5% ovalbumin for 10 h at room temperature. Bovine lactoferrin was labeled with HRPO according to Nakamura et al. (16). After 3 h incubation at room temperature with HRPO-labeled bLf, membrane was thoroughly washed with Tris-buffered saline, pH 7.4, containing .05% Tween-20, and the color reaction was performed using diamino-benzidine as substrate. Standard proteins and proteins from the gel were stained with Coomassie brilliant blue.

RESULTS

A total of 103 *S. aureus* strains isolated from bovine mastitis, grown on blood agar, and tested for bLf binding, demonstrated varying degrees of ^{125}I-labeled bLf binding (Figure 1). Bovine lactoferrin binding was high in 44.7% and moderate in 40.8% of the isolates. Twelve (11.6%) strains showed low binding; 3 strains (2.9%) did not bind bLf. Different growth media were tested to define the optimal cultivation conditions required for staphylococcal cells for bLf binding (Table 1). Cells grown on blood, nutrient, or proteose-peptone agar demonstrated a high bLf binding. However, bLf binding was poor when cells were grown in skim milk or broth media, and binding decreased significantly, in salt or carbohydrate rich media (mannitol salt agar or staphylococcus medium 100). Further characterization of bLf binding was performed on cells grown on blood agar, using a high bLf binding *S. aureus* strain SA-340 (~40% uptake from 8 ng of total ^{125}I-labeled bLf added).

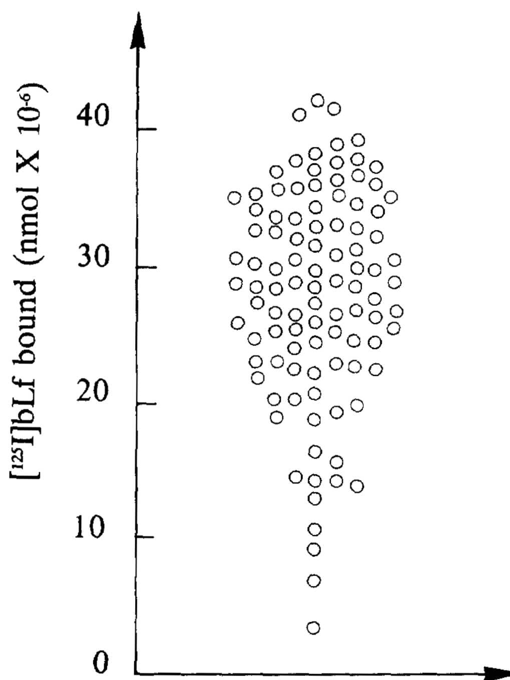

Figure 1. Binding of ^{125}I-labeled bovine lactoferrin (bLf) to *Staphylococcus aureus* strains isolated from acute and chronic bovine mastitis. Cells (10^{9}) of 103 isolates harvested from blood agar were tested. Strains demonstrating protein binding in the range of 10 to 20, 20 to 30, and above 30 × 10^{-6} nmol were considered as low, moderate, and high bLf binders, respectively. Binding below 10 × 10^{-6} nmol was considered insignificant.

TABLE 1. Influence of various culture media on the expression of bovine lactoferrin (bLf) binding in *Staphylococcus aureus* SA-340.

Growth media	^{125}I-labeled bLf bound (nmol × 10^{-6})	
	$\bar{X}$	SD
Blood agar	35.1	1.3
Nutrient agar	25.1	1.2
Proteose-peptone agar	19.9	1.7
Skim milk	11.1	1.5
Tryptic soy agar	10.2	.3
Mannitol salt agar	8.0	.5
Brain-heart infusion agar	5.9	.6
Brain-heart infusion broth	5.8	.3
Nutrient broth	5.3	.1
Staphylococcus medium 110	4.9	1.4

The ^{125}I-labeled bLf binding to cells of SA-340 was stable and showed no dissociation in the presence of high molar NaCl, potassium thiocyanate, or 6 *M* urea. The binding was maximal between pH 4.0 and 7.0. The kinetics of ^{125}I-labeled bLf binding was time dependent, with a requirement of approximately 6 min for 50% and 90 min for 100% saturation (Figure 2). The number of bLf molecules bound per staphylococcal cell was calculated according to Scatchard plot analysis (20). The plot was nonlinear, and the high affinity bLf interaction with SA-340 (association constant, K_a) was approximately 14 × 10^6 L/mol. The number of the saturable receptor sites was estimated at approximately 7200 per cell of SA-340 (Figure 3).

Unlabeled bLf effectively displaced the binding of ^{125}I-labeled bLf to SA-340 in a dose-dependent manner. A concentration of ~6.4 μg of unlabeled bLf caused 50% blocking of ^{125}I-labeled bLf uptake (Figure 4). Various proteins and carbohydrates were tested for a competitive inhibition of ^{125}I-labeled bLf to strain SA-340 (Table 2). Unlabeled hLf (1 mg/ml) caused 65.4% inhibition of ^{125}I-labeled bLf binding. Among other iron-binding proteins used, the inhibitory effect of transferrin was low (28.3%), whereas hemin promoted the ^{125}I-labeled bLf uptake. Plasma glycoproteins, i.e., fibronectin, fibrinogen, and IgG, elicited a low inhibitory effect. Various carbohydrates tested did not inhibit ^{125}I-labeled bLf binding to strain SA-340; however, these compounds enhanced the ligand interaction. The bLf binding components on the cell surface of SA-340 were susceptible to various proteases and carbohydrases (Table 3). Proteinase K treatment of bacterial cells reduced bLf binding to a greater

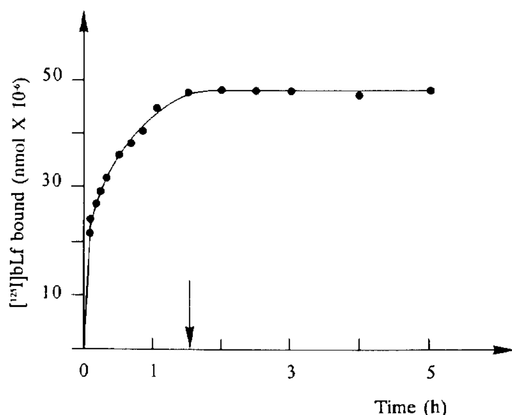

Figure 2. Time course of ^{125}I-labeled bovine lactoferrin (bLf) binding to *Staphylococcus aureus* strain SA-340. Bacteria were incubated with ^{125}I-labeled bLf at time intervals indicated. Values represent the radioactivity bound to 10^9 bacteria after deducting the background (radioactivity) measurement.

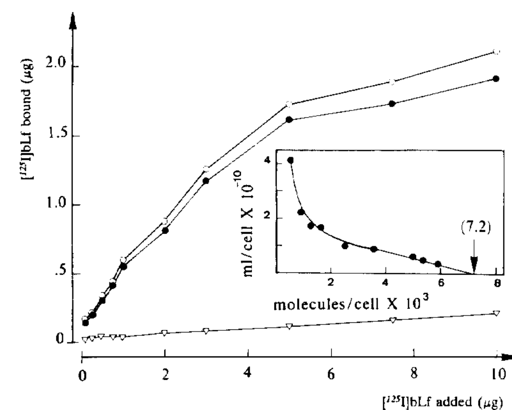

Figure 3. Binding of ^{125}I-labeled bovine lactoferrin (bLf) to *Staphylococcus aureus* strain SA-340 as a function of unlabeled bLf added. Binding of ^{125}I-labeled bLf, in increasing amounts as indicated, to bacteria was performed in the absence (○, total binding) or presence (▽, nonspecific binding) of unlabeled bLf (50-fold excess). Specific binding (●) was calculated by subtracting the nonspecific bLf binding. Inset: Scatchard plot ($r/c = nK_a - rK_a$) of the binding of ^{125}I-labeled bLf to SA-340 (20). The molecules of bLf bound to one bacterial cell (r) and the molecules of bLf free in the medium (c) were calculated using a molecular weight (M_r) of 92,100 for bLf (20, 28). The intercept on X axis represents the number of receptors per cell (n). The slope of the line, represents the effective association constant (K_a) in liters per mole.

TABLE 2. Inhibitory effect of various unlabeled proteins and carbohydrates on ^{125}I-labeled bovine lactoferrin (bLf) binding to *Staphylococcus aureus* strain SA-340.

Inhibitor	Percentage bLf binding inhibition[1]			
	10 µg		100 µg	
	$\bar{X}$	SD	$\bar{X}$	SD
Proteins				
Lactoferrin (bovine)	58.6	.7	88.9	.8
Lactoferrin (human)	48.8	7.2	65.4	6.9
Transferrin	18.6	5.0	28.3	2.2
Hemin	−7.3	3.6	−13.9	2.9
Immunoglobulin G	2.5	2.4	16.7	7.9
Fibronectin	16.1	5.8	32.5	7.4
Fibrinogen	13.6	2.7	29.0	3.6
Fetuin	30.2	.6	37.1	1.4
Carbohydrates				
Glucose	−11.9	1.7	−20.5	4.3
Mannose	−20.2	4.6	−15.2	8.9
Sorbitol	−19.9	6.2	−18.9	7.3
Ribose	−17.2	5.8	−18.0	4.1
Fucose	−18.0	3.3	−17.1	3.8
Galactose	−16.3	1.7	−21.1	2.1
Rhamnose	−15.0	4.3	−14.9	2.6
P-Nitrophenyl-β-D-glucoside	−.8	.2	−10.9	1.1
o-Nitrophenyl-β-D-galactoside	−19.4	2.7	−14.7	4.3
N-Acetyl-D-glucosamine	−8.4	3.8	−12.9	4.3
N-Acetyl-D-galactosamine	−15.7	3.0	−7.9	2.3
N-Acetylneuraminic acid	−8.8	1.9	−2.2	1.9

[1]Inhibition values were calculated as relative percentage of bLf binding to bacteria suspended in phosphate-buffered saline in the absence of any inhibitor.

extent than pepsin or trypsin digestion. Enzyme hydrolysis of bacterial cell surface with neuraminidase impaired the bLf binding more effectively than β-galactosidase. However, α-1-fucosidase treatment did not influence the bLf binding to SA-340. The cell surface components of SA-340 involved in bLf binding were susceptible to both periodate and heat treatment.

The presence of free and functionally active bLf binding components in the lysostaphin digested cell lysate of SA-340 was detected by performing competitive inhibition experiments. The SA-340 cell lysate effectively inhibited the ^{125}I-labeled bLf binding to SA-340 whole cells in a dose-dependent manner (Figure 5). Cell lysate (the pellet and the supernatant after ammonium sulfate precipitation) was tested for the presence of bLf binding components by electrophoretic immunoblotting using HRPO-labeled bLf (Figure 6). Two components with an estimated molecular weight of approximately 92 and 67 kDa were identified as bLf binding receptors. The 92-kDa protein was the major component in the precipitate, and the 67-kDa protein appeared in the supernatant.

DISCUSSION

According to our data, 85% of the mastitis isolates of *S. aureus* demonstrated high or moderate interaction with bLf. The bLf binding to SA-340 was of high affinity with approximately 7200 bLf binding sites per cell. The

TABLE 3. Sensitivity of bovine lactoferrin binding components of *Staphylococcus aureus* strain SA-340 to various enzyme and physico-chemical treatments.

Cell treatment	Percentage decrease in binding	
	$\bar{X}$	SD
Proteases		
Pepsin	78.1	3.9
Trypsin	77.6	1.4
Proteinase K	87.1	1.0
Carbohydrases		
α-1-Fucosidase	6.1	5.5
β-D-Galactosidase	54.7	4.9
Neuraminidase	73.0	4.3
Sodium periodate	58.0	3.3
Heating for 1 h		
50°C	33.8	2.7
80°C	48.8	2.8

binding displacement data suggest that the bacterial cells recognize both labeled and unlabeled forms of bLf and that the binding is reversible. Recently, we have also demonstrated a specific bLf binding mechanism in strains of *Staphylococcus epidermidis*, *Staphylococcus warneri*, *Staphylococcus hominis*, *Staphylococcus xylosus*, *Staphylococcus hyicus*, and *Staphylococcus chromogenes* isolated from bovine intramammary infections (14). The degree of ^{125}I-labeled bLf binding among these coagulase-negative staphylococci was lower than the *S. aureus* strains in the present study. Furthermore, the binding association constants of the above strains ranged between .96 × 10^6 and 11.9 × 10^6 L/mol, values lower than SA-340. Lactoferrin interaction with *S. aureus* is not species specific, because Lf from human milk blocked the ^{125}I-labeled bLf binding. Immunoglobulin G, fibronectin, or fibrinogen did not inhibit (<20%) the ^{125}I-labeled bLf interaction to SA-340, suggesting that the bLf binding component is probably distinct from the staphylococcal cell surface receptors described for the former plasma glycoproteins (15, 22, 27). Other iron-binding proteins such as transferrin or hemin showed little or no interference with ^{125}I-labeled bLf binding to *S. aureus*. Two bLf binding receptors with an estimated molecular weight of approximately 92 and 67 kDa were identified on SA-340. Selective precipitation of the 92-kDa bLf binding component with ammonium sulfate suggests a higher surface hydrophobic nature of this protein compared with 67-kDa bLf binding component identified in the supernatant. Scatchard plot analysis showed nonlinearity, thus the demonstration of two bLf binding components is not unexpected. We have recently isolated a 450-kDa hLf receptor from *S. aureus* associated with toxic shock syndrome. This native receptor has been further resolved into two functionally active components of 62 and 67 kDa (M. Andersson, A. Forsgren, and A. S. Naidu, unpublished data).

Growth conditions may regulate the cell wall and other surface components of staphylococci. *S. aureus* cell surface proteins in the range of 120 to 220 kDa are selectively expressed or enhanced in production when grown on solid media compared with liquid media (4). Our data suggest that the bLf binding property

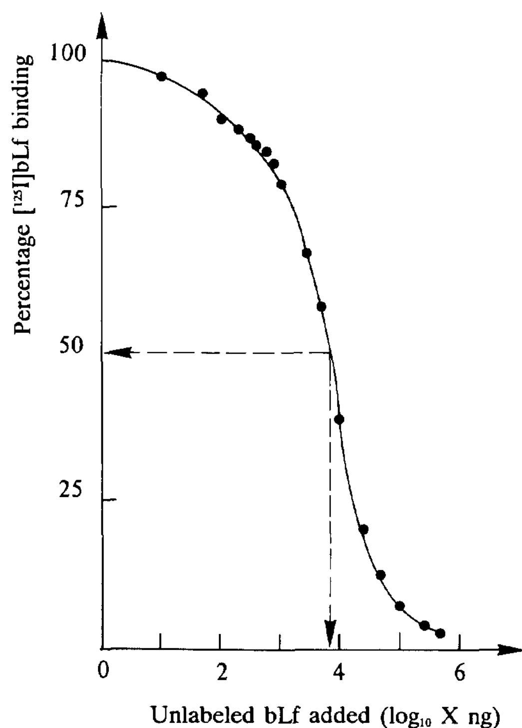

Figure 4. Displacement of ^{125}I-labeled bovine lactoferrin (bLf) binding to *Staphylococcus aureus* strain SA-340 with unlabeled bLf. Approximately 6.4 μg of unlabeled bLf caused 50% displacement of ^{125}I-labeled bLf binding (indicated by arrows).

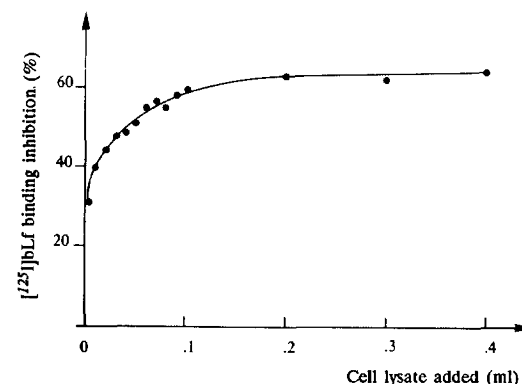

Figure 5. The ^{125}I-labeled bovine lactoferrin (bLf) binding inhibition with *Staphylococcus aureus* strain SA-340 lysate. Increasing amounts of lysate [in .2 ml volumes diluted in phosphate-buffered saline (PBS)] were mixed with approximately 8 ng of ^{125}I-labeled bLf (in .1 ml volumes) and incubated with approximately 10^9 cells (in .1 ml PBS) of SA-340 for 1 h. Cell lysate elicited inhibition of ^{125}I-labeled bLf binding in a dose-dependent manner, suggesting the presence of functionally active bLf binding components in the preparation.

of *S. aureus* is selectively expressed on certain agar media. Carbohydrate- or salt-rich agar growth conditions, known to enhance slime production, have suppressed the binding. Contact of *S. aureus* with milk has been suggested to be critical in the development of intramammary infections (24). The bLf binding capacity of *S. aureus* decreased when grown in skim milk. This effect seems partly due to the stimulated production of proteolytic enzymes by *S. aureus*, which digested the cell surface proteins (12). Furthermore, native bLf present in the milk caused a competitive blocking of the ^{125}I-labeled bLf binding.

Several mammalian receptors mediate glycoprotein clearance through recognition of

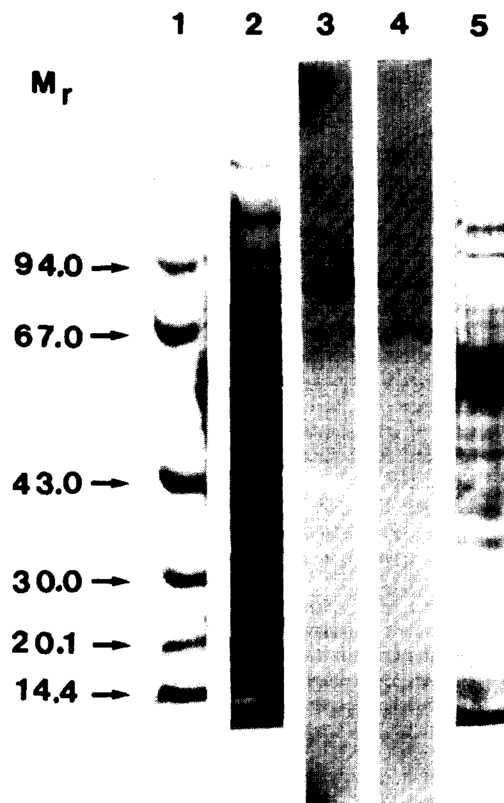

Figure 6. Demonstration of bovine lactoferrin (bLf) binding components of *Staphylococcus aureus* strain SA-340. Lane 1, standard proteins; lane 2, SDS-PAGE of ammonium sulfate precipitated cell lysate; lane 3, Western blot of electrophoretic run from lane 2 with horseradish peroxidase (HRPO)-labeled bLf; lane 4, Western blot of electrophoretic run from lane 5 with HRPO-labeled bLf; and lane 5, SDS-PAGE of supernatant after ammonium sulfate precipitation of the lysate.

different terminal carbohydrate units, in particular, N-acetylglucosamine, mannose, and fucose in the mononuclear-phagocyte system (8). Of the two fucosidyl residues in Lf molecule, the one between α-1-3 linkage with N-acetylglucosamine could be a potential binding determinant (8, 23). Treatment of staphylococci with α-1 fucosidase or incubation of cells with excess of fucose, however, did not cause inhibition of the ^{125}I-labeled bLf uptake. The binding of human adherent mononuclear cells to hLf is mannose-dependent (2); conversely, the bLf binding mechanism to *S. aureus* was mannose-independent. None of the various carbohydrates used in the assay inhibited ^{125}I-labeled bLf binding. The bLf binding component on SA-340 was sensitive to heat or periodate treatment. Enzymatic digestion data on SA-340 evince that staphylococcal bLf binding component contains both a proteinaceous and a glycosidyl moiety in its composition.

Staphylococcus aureus isolated from bovine mastitis bind to fibrinogen, fibronectin, and to various types of collagens present in the bovine udder (10, 12, 27). Such specific interactions may play a role in the adhesion and colonization of staphylococci to the epithelial surface. In a recent study, we have shown that the bLf binding property is associated with fibronectin, fibrinogen, and laminin receptors in *S. aureus* strains belonging to capsular polysaccharide serotypes CP-5 and CP-8. However, no correlation was found between bLf binding and the expression of protein A (A. S. Naidu, J. M. Fournier, S. Kalfas, J. L. Watts, and A. Forsgren, unpublished data). It has been established that CP-5 and CP-8 are the most prevalent of the total 11 capsular serotypes among *S. aureus* mastitis strains (17).

Lactoferrin has an important role both in the antigen processing and antibody producing cellular pathways. From our binding and inhibition data, it seems that the Lf binding mechanism to *S. aureus* is distinct from Lf binding to monocytes, macrophages, and PMN. Importantly, Lf is also an antimicrobial substance; however, its adsorption to the bacterial cell surface is a prerequisite for eliciting bactericidal effects (5). However, the role of this specific bLf-staphylococcal interaction in mediating bactericidal effects or in processing of bacteria by cells of reticuloendothelial system remains to be elucidated.

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