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Binding of Laminin to Oral and Endocarditis Strains of Viridans Streptococci

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Attachment of bacteria to the host tissue is regarded as a crucial step in the development of many types of infections. Recent studies by us and others have shown that matrix proteins which serve as adhesion proteins for eucaryotic cells may also be recognized by some bacteria. In the present communication, we report that several strains of viridans streptococci are able to bind to laminin. Most strains isolated from blood and heart valves of patients with endocarditis expressed laminin receptors, whereas only a few of the strains isolated from the oral cavity recognized this protein. This observation indicates that laminin binding might be an important factor in the pathogenesis of viridans endocarditis. Laminin binding to two strains (*Streptococcus mitis* UAB594 and UAB597) isolated from patients with endocarditis was characterized further. The bacterial cells expressed a limited number of laminin receptors (4×10^2 to 1×10^3 per cell) which bound the protein in a high-affinity interaction (K_d, 40 to 80 nM). This receptor of *S. mitis* UAB594 was heat labile and could be solubilized from bacteria by brief digestion with trypsin. Solubilized receptors which competed with cell-bound receptors for ^{125}I-laminin could be adsorbed on laminin-Sepharose but not on Sepharose substituted with fibrinogen or fibronectin. Comparison of laminin receptors from *S. mitis* with those previously described for *Streptococcus pyogenes* suggest that different sites in the laminin molecule are recognized by the two bacteria and hence that the corresponding receptor molecules are not identical.

Viridans streptococci constitute a heterogeneous group of bacteria colonizing various ecological niches within the oral cavity. For example, *Streptococcus salivarius* and *S. mitior* colonize mucosal surfaces, while *S. mutans* and *S. sanguis* are isolated mostly from pellicle-coated tooth surfaces (13, 14). The molecular mechanisms mediating the attachment of viridans streptococci to oral epithelium or to mineralized tissues are incompletely understood despite intensive investigation, particularly on the role of *S. mutans* in the etiology of dental caries.

Viridans streptococci are the most prevalent group of bacteria causing subacute bacterial endocarditis. *S. sanguis* strains have been isolated from 30 to 40% of diagnosed cases (2, 6, 22). The factors that enable the usually noninvasive inhabitants of the oral cavity to cause subacute bacterial endocarditis are unknown. Viridans streptococcal bacteremias frequently follow dental manipulations and abrasions of the gums. They lead only occasionally to colonization and infection of the endocardium. Two factors can contribute to the development of the clinical symptoms of endocarditis—bacterial virulence and altered host resistance factors (for a review, see reference 3). A high incidence of endocarditis can be correlated with preexisting heart problems and the presence of damaged heart valves. Injury and disease may alter the nonadhesive, nonthrombogenic surface of healthy heart valves, exposing components which may induce platelet aggregation and thrombus formation. In fact, in subacute bacterial endocarditis thrombi are commonly found on the surface of heart valves. They might serve as a substrate for the adhesion of viridans streptococci, as proposed by Herzberg et al. (10, 11). In the present study, we analyzed the possibility that viridans streptococci may attach directly to subendothelial matrix components.

Laminin is a constituent of the basement membrane underlying the epithelium and is not normally exposed in undamaged tissues except in basement membranes underlying fenestrated vascular endothelium such as kidney glomeruli (1, 17). Presumably it can be exposed after the tissue has been damaged by mechanical trauma or by an inflammatory process or after lysis of the epithelium by bacterial toxins. We have previously postulated that laminin in basement membranes may serve as an attachment protein that is recognized by bacteria in the colonization of the host tissue. Some bacteria—*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Treponema pallidum*—have been demonstrated to possess components on their surface that specifically recognize and bind to laminin (9, 16, 25, 26).

In this investigation we have screened oral and endocarditis strains of viridans streptococci for the ability to bind laminin. This interaction was characterized further for selected strains.

MATERIALS AND METHODS

Chemicals. Laminin was isolated from the mouse Engelbreth-Holm Swarm sarcoma tumor as described (28), and fibronectin was purified from human plasma by affinity chromatography or gelatin-Sepharose (5, 19). Fibrinogen, human immunoglobulin G (IgG), fetuin, bovine serum albumin, and trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone treated, 120,000 U/mg) were from Sigma Chemical Co., St. Louis, Mo. Proteins were labeled with ^{125}I (specific activity, 15 mCi/mg; Amersham Corp., Arlington Heights, Ill.) by the chloramine T method (13).

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TABLE 1. Bacterial strains[a]

Species and strain	Serotype	Plasmid	Source	Relative ^{125}I-laminin binding[b] (% of control)
S. mutans				
HS 6	a	−	T. Shiota	6.1
BHT	b	−	T. Shiota	8.1
PS 14	c	−	H. Murchison	12.4
V 318	c	+	F. Macrina	6.9
UAB 305	c, e, or f	+	H. Murchison	11.1
UA 101	c, e, or f	+	P. Caufield	5.9
UA 130	c, e, or f	−	P. Caufield	7.2
UA 159	c, e, or f	−	P. Caufield	4.2
UA 174	c, e, or f	+	P. Caufield	11.4
OMZ 176	d	−	T. Shiota	9.5
LM 7	e	+	D. Clewell	18.5
OMZ 175	f	−	T. Shiota	7.0
6715	g	−	H. Murchison	3.7
S. mitis 3 W		−	J. McGhee	32.8
S. mitior SS 429		−	J. McGhee	120.9
S. sanguis				
Challis		+	D. Behnke	3.4
10556		ND[c]	ATCC[d]	57.2
S. salivarius 13419		ND	ATCC	38.0
S. anginosus DL 8		ND	D. LeBlanc	1.5
S. faecalis 19433		−	ATCC	6.4
S. faecium 19434		−	ATCC	17.0
S. durans 19432		−	ATCC	24.0
S. equinis 9812		−	ATCC	23.1
S. lactis 19435		−	ATCC	18.1
S. mutans				
SS 1134		−	J. Washington III	21.3
SS 1122		−	R. Facklam	23.8
31756		−	R. Facklam	108.2
0829676		−	J. Washington III	10.1
3078791		+	J. Washington III	79.5
1486346		−	J. Washington III	14.2
1797917		−	J. Washington III	22.2
S. mitis				
UAB 594		−	W. Dismukes/G. Cobbs	115.3
UAB 597		−	W. Dismukes/G. Cobbs	33.2
S. sanguis				
I UAB 593		−	W. Dismukes/G. Cobbs	32.8
I UAB 595		−	W. Dismukes/G. Cobbs	43.5
I UAB 599		−	W. Dismukes/G. Cobbs	107.1
II UAB 596		−	W. Dismukes/G. Cobbs	50.1
S. MG-intermedius				
UAB 596		−	W. Dismukes/G. Cobbs	94.3
UAB 598		−	W. Dismukes/G. Cobbs	4.3

[a] All *S. mutans* strains were originally isolated from dental plaque or carious lesions except those supplied by J. Washington III and R. Facklam, which were isolated from patients with endocarditis. Strains UAB593 through UAB600 also came from patients with endocarditis. Other streptococci came from oral, fecal, or nonhuman ecological niches.

[b] Determined as described in the legend to Fig. 1.

[c] ND, Not determined.

[d] ATCC, American Type Culture Collection.

Bacteria. Strains of viridans streptococci originated from collections at the University of Alabama at Birmingham (Table 1). Classification of bacteria was based on two reference systems (7, 8), by fermentation of mannitol, sorbitol, inulin, lactose, raffinose, and sucrose and hydrolysis of arginine and esculin. Strains were maintained in brain-heart infusion broth (Difco Laboratories, Detroit, Mich.) containing 10% glycerol at −20°C. Bacteria were subcultured on blood agar plates and grown in brain-heart infusion medium for 18 h to early stationary phase at 37°C. Bacteria were harvested by centrifugation at 1,350 × *g* for 15 min, washed with phosphate-buffered saline (PBS; 0.14 M sodium chloride, 10 mM phosphate, 0.02% sodium azide, pH 7.4), and suspended in PBS. In a series of experiments, bacteria were also grown in Todd-Hewitt broth (Difco) and a chemically defined medium (27). The bacterial suspensions could be stored frozen at −20°C for several weeks without loss of binding activity. The binding of ^{125}I-laminin to viridans streptococci was compared with that of *S. pyogenes* 1321, which has been characterized previously (26). The latter was grown in Todd-Hewitt broth as described above for strains of viridans streptococci, but the washed cells were heat-killed for 10 min at 80°C. This procedure did not result in loss of ^{125}I-laminin-binding activity. The number of bacteria was determined for each strain by counting cells in a Petroff-Hausser chamber. The number of bacteria in the suspensions of *S. mitis* and *S. pyogenes* was routinely calculated from a previously prepared standard curve, relating absorbance at 600 nm to the number of bacteria counted in the Petroff-Hausser chamber.

Binding of ^{125}I-laminin to bacteria. The binding of ^{125}I-laminin to cells was quantified essentially as described previously (23). If not indicated otherwise, 2×10^9 bacteria were incubated with 5×10^4 cpm (ca. 80 ng) of the radiolabeled laminin in PBS containing 0.1% bovine serum albumin in a total volume of 0.5 ml. The tubes containing the incubation mixtures were fitted onto a mixer with a rotating axis and incubated end-over-end at 20°C for 1 h unless otherwise stated. Subsequently, 400 μl of the incubation mixture was carefully added to 0.5 ml of PBS layered on top of a Percoll solution in PBS (density, 1.02 g/ml). The samples were centrifuged at 1,350 × *g* for 15 min in a swinging-bucket rotor. The supernatant fluid was removed by aspiration, and the radioactivity associated with the bacterial pellet was quantified in a gamma counter (LKB Wallac, Turku, Finland). Radioactivity recovered from incubation mixtures containing no bacteria was considered to be background and subtracted. The plastic tubes used in the experiments were precoated by overnight incubation with 0.1% bovine serum albumin in PBS to minimize nonspecific binding of bacteria and proteins to the walls of the tubes.

Digestion of bacteria with trypsin. Cell wall components of viridans streptococci and *S. pyogenes* were solubilized by treatment of cells with trypsin. Cells (200 mg, wet weight) were suspended in PBS without azide and digested with 25 μg of trypsin per ml for 1 h at 37°C or as otherwise stated. The reaction was stopped by the addition of soybean trypsin inhibitor (50 μg/ml). Cells were pelleted by centrifugation, and supernatant fluids were stored at −20°C.

Immunization. Polyclonal antibodies against laminin were raised in New Zealand white rabbits by three successive intramuscular injections of 50 μg of laminin emulsified in incomplete Freund adjuvant (Difco) at 10-day intervals. Rabbits were bled 10 days after the last injection and subsequently bled four times at 10-day intervals.

The IgG fraction of the serum was purified on a column of protein A-Sepharose (Pharmacia, Uppsala, Sweden), and absorbed antibodies were eluted with 3 M $MgCl_2$ (12), dialyzed against PBS, and stored frozen in small portions. The purified IgG was absorbed with bacteria. Purified IgG (200 μg) was incubated with 200 μg (wet weight) of bacteria (*S. mitis* UAB594) in 1 ml of PBS for 1 h at 37°C and overnight at 4°C. The mixture was centrifuged (3,000 × *g*, 15 min), and the supernatant was stored at −20°C until used.

RESULTS

Screening of viridans streptococci for binding of laminin and other matrix proteins. Many pathogenic bacteria have recently been shown to possess specific receptors for proteins of the extracellular matrix, such as fibronectin, laminin, fibrinogen, and collagen. The binding of bacteria to the extracellular matrix seems to represent a mechanism of bacterial attachment to host tissues. We initially tested several strains of viridans streptococci for the ability to bind selected matrix proteins that could conceivably mediate the attachment of bacteria to the damaged heart tissue. Among the strains tested, none bound ^{125}I-labeled derivatives of fibronectin or fibrinogen, but some bound ^{125}I-laminin. This prompted us to screen a larger collection of 39 strains and characterize this binding.

Several strains of viridans streptococci were found to bind ^{125}I-laminin (Fig. 1 and Table 1). Our collection included randomly selected strains of streptococci isolated from the oral cavity of healthy individuals as well as strains isolated from the blood of patients with clinical symptoms of endocarditis. While most of the oral strains bound no or low amounts of laminin, strains obtained from patients with endocarditis mostly bound medium to high levels of labeled protein. Hence, the average level of binding by strains isolated from patients with endocarditis was three times higher than that of oral strains (Fig. 1). However, a few of the strains isolated from the oral cavity bound laminin, and the range of binding was similar in both groups.

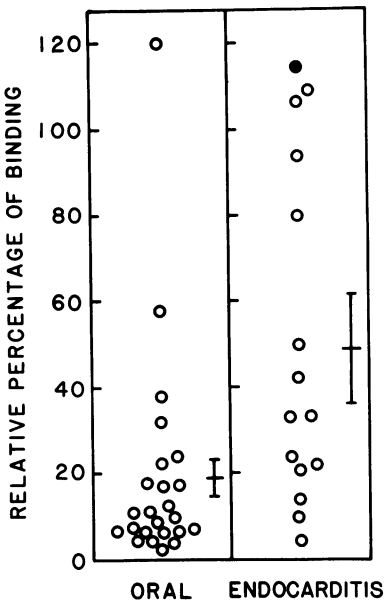

FIG. 1. Binding of ^{125}I-laminin to various strains of viridans streptococci isolated from either the oral cavity of healthy individuals or the blood of patients with endocarditis. To compare binding data obtained in different experiments, cells of a batch of *S. pyogenes* 1321 were included at each experimental condition and the amounts of ^{125}I-laminin bound to the different strains were expressed relative to that bound to *S. pyogenes* (set at 100%). Typically, 2×10^9 cells of *S. pyogenes* bound 1.6×10^4 cpm of ^{125}I-laminin when 5×10^4 cpm of labeled protein of indicated specific activity was added to the incubation mixture. Each circle indicates one strain; bars indicate the mean values (±SE) of both populations. Strains were assayed for binding after 1 h of incubation as described in Materials and Methods. *S. mitis* UAB594 is indicated with a solid circle. To assess the reproducibility of these results, a handful of strains stored at −20°C for several months were thawed and cultured. Their relative binding potential remained unchanged.

Bacteria expressed the laminin-binding components to a higher extent when grown in brain-heart infusion broth than when grown in Todd-Hewitt broth or chemically defined medium (27), although all media were sufficient to support bacterial growth.

Characterization of laminin binding to *S. mitis* UAB594 and UAB598. One of the high-binding endocarditis strains, *S. mitis* UAB594, as well as *S. mitis* UAB597, binding moderate amounts of ^{125}I-laminin, were selected for further characterization of the bacteria-laminin interaction. Binding of ^{125}I-laminin to cells of both strains was rapid and essentially complete within 1 h of incubation. The amount of bound protein remained constant when incubation was prolonged (Fig. 2). In subsequent experiments the incubations were routinely carried out for 1 h to allow maximal binding. Binding by cells of strain *S. MG-intermedius*, considered a nonbinder, was consistently low and did not exceed 5% of the values reported for *S. mitis* UAB594.

The binding of laminin to bacteria was reversible, i.e.,

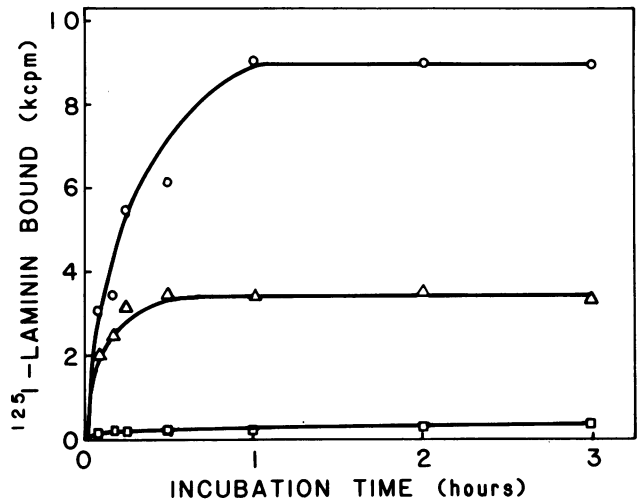

FIG. 2. Time course of binding of ^{125}I-laminin to cells of three strains of viridans streptococci: *S. mitis* UAB594 (○), *S. mitis* UAB597 (△), and *S. MG-intermedius* UAB598 (□). In this experiment, unbound ^{125}I-laminin was separated from bacteria by rapidly diluting incubation mixtures with 3 ml of ice-cold PBS followed by centrifugation. This method of separation allows more accurate assessment of the amount of ^{125}I-laminin bound for very short incubation times. Data refer to the amounts of radioactivity bound by 2×10^9 bacterial cells. Background values were subtracted.

incubation of bacteria containing bound ^{125}I-laminin with excess amounts of unlabeled laminin resulted in displacement of the bound ^{125}I-labeled protein. Excess amounts of unlabeled fibrinogen or fibronectin did not displace bound ^{125}I-laminin (data not shown).

The composition of labeled material bound to bacteria was analyzed by polyacrylamide gel electrophoresis. Under reducing conditions, two polypeptides with the migration properties of the A and B chains of laminin (4) were associated with bacteria (data not shown).

The specificity of binding was further studied in direct competition experiments in which ^{125}I-laminin was added to the bacteria together with a large amount (1,000- to 5,000-fold excess) of unlabeled proteins (Table 2). Unlabeled laminin inhibited the binding of the ^{125}I-labeled derivative in a dose-dependent manner. Other proteins tested were not as effective inhibitors of ^{125}I-laminin binding, although some proteins (fetuin and fibrinogen) inhibited the binding of ^{125}I-laminin substantially. The molecular mechanisms behind the unobserved inhibition by these unrelated proteins remain unclear.

The specificity of the binding of laminin to *S. mitis* UAB594 was also analyzed in experiments with antibodies against laminin. To minimize interactions between serum components and bacteria, which could nonspecifically affect laminin binding, the IgG fraction of serum was purified and further absorbed with bacteria (*S. mitis* UAB594). These purified, preabsorbed fractions of immune and preimmune IgG were used in inhibition experiments.

Preincubation of ^{125}I-laminin with antilaminin IgG inhibited the binding of the labeled protein to bacteria in a concentration-dependent manner, whereas nonimmune IgG from the same rabbit had little effect (Fig. 3). Since IgG itself does not interfere with binding of ^{125}I-laminin to bacteria (Table 2), it seems reasonable to assume that the antibodies block the regions in the laminin molecule which are recognized by bacterial receptors.

TABLE 2. Inhibition of ^{125}I-laminin binding to *S. mitis* UAB594 by unlabeled proteins[a]

Protein	% Inhibition (mean ± SE)	
	100 μg	500 μg
No addition	0	0
Laminin	64.7 ± 11.4	94.2 ± 10.1
Fibrinogen	19.9 ± 3.2	38.7 ± 5.6
Fibronectin	−3.0 ± 1.3	2.4 ± 1.1
IgG	−2.8 ± 1.5	11.3 ± 3.7
Fetuin	8.1 ± 3.1	25.7 ± 3.9
Ovalbumin	6.6 ± 2.0	6.3 ± 2.9
Orosomucoid	3.8 ± 1.1	4.3 ± 2.8

[a] Cells of *S. mitis* UAB594 (2×10^9) were incubated for 3 h with a mixture of 10^4 cpm of ^{125}I-labeled laminin (ca. 50 ng) and 100 or 500 μg of unlabeled protein. Controls were assayed in the absence of proteins.

Quantitation of binding as a function of increasing concentrations of ^{125}I-laminin showed that bacterial cells could be saturated with labeled ligand (Fig. 4), which suggests that this interaction involves a limited number of bacterial receptors. A maximum of 1.5 μg of laminin bound to 10^9 cells of *S. mitis* UAB594. If we assume that all laminin associated with the streptococcal cells represents receptor-bound ligand and that the molecular weight of laminin is 90,000 (4), the average number of receptors per cell of *S. mitis* UAB594 is 10^3.

Similar quantitative analysis of laminin binding for *S. mitis* UAB597 revealed the presence of 4×10^2 receptors. For both strains, Scatchard analysis (24) of binding data (Fig. 5) revealed straight lines, which suggests that each bacterial strain possesses only one class of receptor site. The calculated K_d values for the binding of laminin to *S. mitis* UAB594 and UAB597 are 80 and 40 nM, respectively.

Laminin binding components of *S. mitis* UAB594. Recent results (26) indicated that the laminin-binding components from the cell wall of *S. pyogenes* are heat-stable proteins, which can be released by digestion of bacteria with trypsin. Therefore we investigated the possibility that a similar component is responsible for binding of laminin to cells of *S. mitis* UAB594.

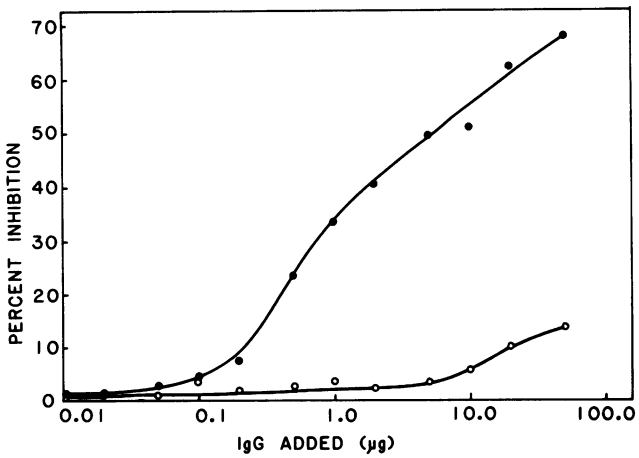

FIG. 3. Inhibition of binding of ^{125}I-laminin to *S. mitis* UAB594 by antibodies against laminin. ^{125}I-laminin (10 ng, 5×10^4 cpm) was preincubated with indicated amounts of absorbed antilaminin rabbit immune (●) or preimmune (○) IgG for 1 h before the addition of bacteria to the assay mixture. After 1 h of incubation, the amount of ^{125}I-laminin bound to bacteria was determined as indicated in the text.

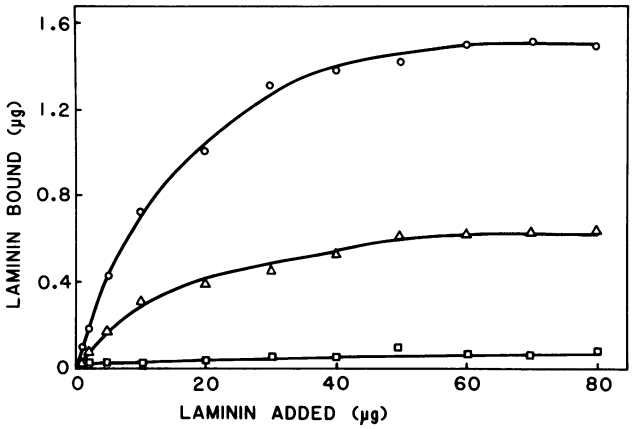

FIG. 4. Saturability of binding of ^{125}I-laminin to *S. mitis* UAB594 (○), *S. mitis* UAB597 (△), and *S. MG-intermedius* UAB598 (□). Bacteria were incubated in the presence of increasing concentrations of labeled laminin. Data refer to the amount of laminin bound by 10^9 bacteria. Background values were determined for each concentration of added laminin and subtracted for the incubation mixtures containing bacteria.

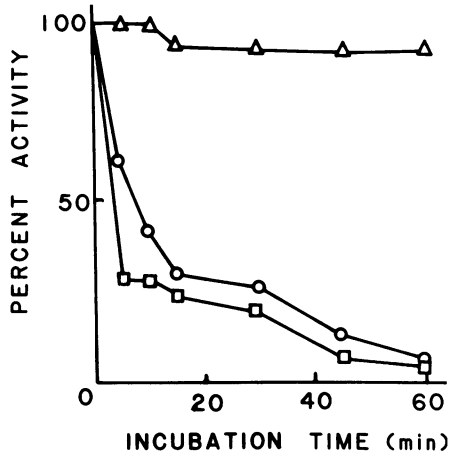

FIG. 6. Temperature sensitivity of laminin-binding components of *S. mitis* UAB594. Bacteria were incubated at 60°C (△), 80°C (○), or 100°C (□) for the indicated periods of time prior to incubation with ^{125}I-laminin. Data are expressed as percent binding relative to unheated bacteria.

Bacterial cells were heated at various temperatures, and their ability to bind ^{125}I-laminin was analyzed. Binding decreased dramatically for cells heated at 100 or 80°C, whereas bacteria incubated at 60°C largely retained their ability to bind laminin (Fig. 6). Thus, the laminin-binding components of this strain appear to be more heat labile than the laminin receptors of *S. pyogenes*, which withstand heating for 10 min at 100°C with only marginal loss of activity.

The laminin-binding component of *S. mitis* UAB594 could be released from cells by trypsin digestion (Fig. 7). Release of receptors by trypsin was rapid, as indicated by a significant reduction in ^{125}I-laminin binding of treated cells. Components capable of inhibiting the binding of ^{125}I-laminin to bacteria were detected in the tryptic digest. However, the increase in the inhibitory activity of the supernatant did not follow the same kinetics as the release of laminin-binding components from cells. More than half of the measurable receptors were released from bacterial cells by trypsin after 5 min of incubation, whereas the peak of the inhibitory activity was observed after 1 h of incubation. Incubations carried out for a longer time resulted in reduced inhibitory activity, presumably as a result of degradation of active components by proteases. These findings may indicate that the accessibility of the receptors to trypsin may be a limiting step of the receptor solubilization process.

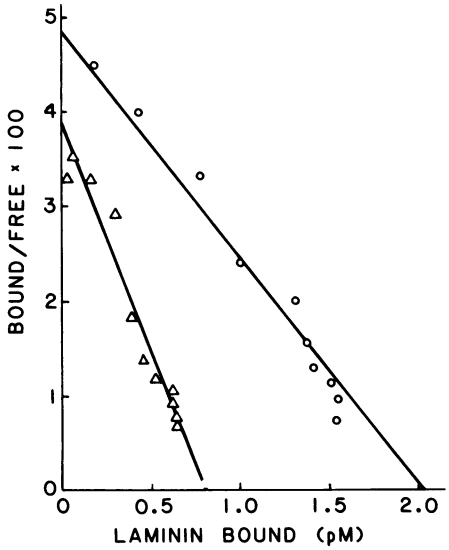

FIG. 5. Scatchard plot (24) analysis of the data presented in Fig. 5 after subtraction of values for unspecific binding to receptor-negative strain *S. mitis* UAB598. Symbols: ○, *S. mitis* UAB594; △, *S. mitis* UAB597. The binding data for kinetic analysis for calculated by a nonweighted linear regression computer program.

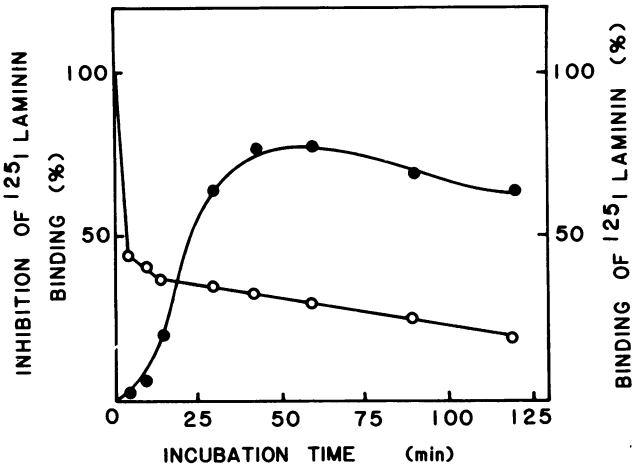

FIG. 7. Solubilization of laminin-binding components of *S. mitis* UAB594 by trypsin. Bacteria were digested with trypsin (see Materials and Methods), and digested bacterial cells were assayed for binding activity (○). Inhibition of ^{125}I-laminin binding to intact cells of *S. mitis* UAB594 by 200 μl of enzyme-released material was also determined (●). The amount of ^{125}I radioactivity recovered in the tube in the absence of trypsin-released material after subtraction of the background value was set as 0% inhibition, and all data are expressed as percentage of control.

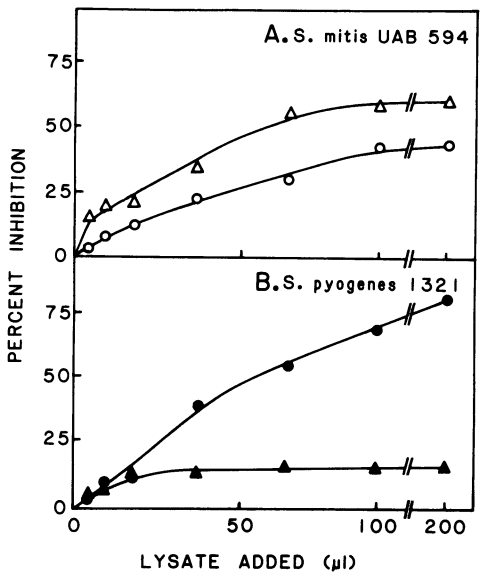

FIG. 8. Inhibition of laminin binding to bacteria by solubilized receptors. Laminin receptors solubilized from *S. pyogenes* 1321 (○, ●) or *S. mitis* UAB594 (△, ▲) by trypsin treatment were tested as inhibitors of ^{125}I-laminin binding to *S. mitis* UAB594 (A) or *S. pyogenes* 1321 (B). One hundred microliters of enzyme-related material equals the amount solubilized from 20 μg (wet weight) of bacteria. Data are expressed as percentages of inhibition, where binding to bacteria incubated in the absence of potential inhibitors was set as 0% inhibition.

We have previously reported that a tryptic digest of *S. pyogenes* cells contains active receptor fragments that can inhibit the binding of ^{125}I-laminin to intact cells of *S. pyogenes*. To examine the relationship of laminin receptors from *S. pyogenes* and *S. mitis*, material released by trypsin digestion from *S. mitis* UAB54 and *S. pyogenes* 1321 was used in cross-inhibition experiments. Binding of ^{125}I-laminin to cells of *S. pyogenes* 1321 was substantially inhibited by the trypsin-released proteins from the same strain, but to a much lower extent by a similar digest from *S. mitis* UAB594 (Fig. 8B). In contrast, binding of ^{125}I-laminin to *S. mitis* cells was inhibited to a similar extent by trypsin digests of both strains (Fig. 8A). In control experiments, soybean trypsin inhibitor-trypsin was added to incubation mixtures. The inactivated trypsin did not interfere with the binding of ^{125}I-laminin by either strain.

This difference was further confirmed in experiments in which laminin-binding components were absorbed by affinity chromatography. Trypsin digests of both strains were checked for inhibitory activity after passage through columns of unsubstituted Sepharose gel or Sepharose gels substituted with laminin, fibrinogen, or fibronectin. Passing trypsin digests of strain *S. pyogenes* 1321 through laminin-Sepharose resulted in removal of most of the inhibitory activity (Table 3). Also, when the trypsin digest of *S. mitis* was passed through laminin-Sepharose, the ability to compete for ^{125}I-laminin with intact cells of the same strain was markedly reduced. Surprisingly, the same lysate passed through a column of laminin-Sepharose retained its low inhibitory activity towards cells of *S. pyogenes* 1321. These data suggested that the ability of a trypsin digest of *S. mitis* to partially inhibit the binding of ^{125}I-laminin to *S. pyogenes* may not be due to solubilized laminin receptors.

Passing the trypsin digests through columns of unsubstituted Sepharose or Sepharose substituted with fibrinogen or fibronectin did not affect their inhibitory activity.

DISCUSSION

Infective endocarditis has received considerable attention for the past century. Proper therapy markedly improves the prognosis of the disease, which was almost universally fatal before the antibiotic era. It is not known why cardiac valves and not the other parts of the heart or vascular system are colonized by bacteria or why some species of *S. viridans* cause endocarditis while other species only cause bacteremia. It is also unclear how strains of viridans streptococci which are usually noninvasive in the oral cavity colonize and invade heart epithelium. The different colonizing potential of various species and strains of viridans streptococci may be related to their different abilities to interact with extracellular matrix components in the heart valves. Our observation that laminin, a basement membrane component, is recognized by bacteria points to the possibility that this interaction mediates bacterial attachment to host tissues.

For two of the endocarditis strains, *S. mitis* UAB594 and UAB597, the binding of laminin was characterized further. Cells of both strains bound laminin rapidly. The number of laminin-binding sites was limited and varied with growth conditions, as has been observed for other bacterial receptors for connective tissue proteins (20, 26).

The number of laminin receptors, 10^3 and 4×10^2 for *S. mitis* UAB594 and UAB597, respectively, is comparable to that of *S. pyogenes* (10^3), but much lower than the number of available receptors on the surface of eucaryotic cells. Mammalian cells were reported to contain 10^4 to 10^5 laminin receptors (18, 21). Taking into consideration the larger size of eucaryotic cells, the density of receptors appears to be as high or higher on bacterial surfaces as on eucaryotic cell surfaces. The affinity of bacteria for laminin seems to be of the same order of magnitude as that of eucaryotic cells.

The heat-labile components responsible for binding of laminin could be released from the cell walls of *S. mitis* UAB594 by treatment with trypsin. This solubilized material was active in that it competed with intact bacterial cells for ^{125}I-laminin.

TABLE 3. Absorption of inhibitory activity from trypsin digests of *S. pyogenes* 1321 and *S. mitis* UAB594 by affinity chromatography[a]

Inhibitor	% Inhibition of ^{125}I-laminin binding to:	
	S. pyogenes	*S. mitis*
None	0	0
Trypsin digest of *S. pyogenes*		
Unfractionated	68.8	62.3
Passed through laminin-Sepharose	10.1	2.7
Trypsin digest of *S. mitis*		
Unfractionated	21.9	69.3
Passed through:		
Sepharose CL4B	21.9	70.4
Laminin-Sepharose	19.7	15.2
Fibronectin-Sepharose	22.4	64.9
Fibrinogen-Sepharose	20.6	68.3

[a] Solubilized cell wall components of both strains were passed through columns of Sepharose (bed volume, ca. 1 ml), and 300 μl of each untreated or absorbed material was checked for inhibitory activity in the standard assay. The amount of ^{125}I radioactivity recovered in the absence of trypsin digestion after subtraction of the background value was set as 100% activity, i.e., 0% inhibition, and all data are expressed as percentages of the control.

Laminin receptors of *S. mitis* UAB594 differ from those of *S. pyogenes* in that they appear to recognize different sites in the laminin molecule. A trypsin digest of *S. mitis* inhibited the binding of ^{125}I-laminin to *S. mitis* but only weakly inhibited binding to *S. pyogenes*. Furthermore, the weak inhibitory activity in the *S. mitis* digest directed to *S. pyogenes* was not absorbed on laminin-Sepharose and was presumably not due to the presence of solubilized receptor. On the other hand, a trypsin digest of *S. pyogenes* inhibited binding of ^{125}I-laminin to both *S. pyogenes* and *S. mitis*. Our previous studies (26) have shown that the solubilized laminin receptors from *S. pyogenes* are large molecules (M_r > 30,000). Perhaps the binding sites on the laminin molecule for *S. pyogenes* and *S. mitis* are closely spaced so that binding of *S. pyogenes* receptors to ^{125}I-laminin sterically interferes with the binding of the protein to *S. mitis*.

Binding of bacteria to laminin exposed after damage of heart valve endothelium may represent a mechanism of adherence. This study indicates that only some strains of oral streptococci have the potential to bind laminin, whereas most of the strains isolated from patients with endocarditis bind laminin. Therefore, it does not seem unreasonable to propose that the ability to bind laminin may be one adhesive mechanism crucial in the development of the disease and that oral strains that bind laminin are potentially capable of colonizing heart valves.

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