AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 20, NUMBER 2, JUNE 1991

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne
Berlin Paris Vienna

ISSN 0189-2913

Afr. J. Med. med. Sci. (1991) **20**, 89–95.

Influence of mucin on serum and connective tissue protein binding to *Staphylococcus aureus* isolated from nasal carriage and clinical sources

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Summary

A total of 89 *Staphylococcus aureus* strains were tested for [^{125}I]-labelled fibronectin (Fn), type I (Cn-I), type II (Cn-II), type IV (Cn-IV) collagens and laminin (Lm) binding, and nasal carriage isolates (54 strains) demonstrated higher degree of interaction than clinical isolates (35 strains). Strains belonging to nasal carriage group, after preincubation with mucin demonstrated a significant decline in binding to Fn (39.4%), Cn-I (44.7%), Cn-IV (42.0%) and Lm (43.5%) compared with inhibition of binding of clinical isolates to Fn (13.3%), Cn-I (8.0%), Cn-IV (9.8%) and Lm (11.2%). *S. aureus* strain Nig-6 demonstrated a mucin concentration (in the range 0.01 to 100 mg/ml) dependent decrease of [^{125}I]-labelled serum and connective tissue protein binding. Mucin concentrations of 100, 150, 175 and 250 µg/ml when incubated with 10^9 cells, caused 50% displacement of [^{125}I]-labelled Lm, Cn-I, Cn-IV and Fn uptake respectively. Mucin interaction with bacterial cells seems probably important in the pathomechanism of staphylococcal adhesion and colonization.

Résumé

L'examen de 89 variantes de *Staphylococcus aureus* pour déterminer l'ancrage de la fibronectine (Fn), des collagènes Type-I (Cn-I), II (Cn-II), IV (Cn-IV) et de laminine (Lm) libellées à l'iode 125 a montré que les échantillons des parois nasales (54 variantes) accusent une plus forte incidence d'interaction que les échantillons cliniques (35 variantes). Les variantes issues des parois nasales ont marqué, après incubation à la mucine, une baisse sensible de la capacité à s'ancrer au Fn (39.4%), Cn-I (44.7%), Cn-IV (42.0%) et Lm (43.5%) par rapport à l'inhibition à l'ancrage des échantillons cliniques au Fn (13.3%), Cn-I (8.0%), Cn-IV (9.8%) et Lm (11.2%). La variante Nig-6 de *S. aureus* connait une baisse de concentration de mucine (de 0.01 à 100 mg/ml) reflèteé dans l'ancrage du sérum libellé à l'iode 125 et de la protéine dans le tissu connexe. Une concentration de mucine de 100, 150, 175 et 250 µg/ml, après incubation avec des cellules 10^9, a provoqué un déplacement, à 50%, de, respectivement, Lm, Cn-I, Cn-IV libellés à l'iode 125 et l'aération Fn. L'interaction de la mucine avec les cellules bactériennes semble indispensable au pathomécanisme de l'adhésion et la colonisation du staphylocoque.

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Introduction

Mucosal surface is the primordial target for most of the microbial infections. The interface between mucosae and the external environment comprises mucins and the complex hydrated gel-like secretions of mucosae or of some exocrine glands [1,2]. Mucins display a structural and compositional heterogeneity in carbohydrate chains, providing an important function in the mucosal colonization of microbial pathogens and commensals [1]. Mucins also enhance the mucosal barrier function by serving as a binding matrix for other biological substances, such as lactoferrins, immunoglobulins, serum glycoproteins and lipids [3,4].

Interaction of several bacterial pathogens with mucins has been demonstrated. *Escherichia coli*, causing intestinal or urinary tract infections, binds to mucins at the respective tissue mucosal surface [5]. Tracheobronchial mucins have specific sialic acid receptors for *Pseudomonas aeruginosa* [6].

Staphylococcus aureus causes various illnesses among humans and animals, ranging from mild superficial to acute systemic infections [7]. Specific binding of various serum and connective tissue proteins to staphylococci has been implied in the pathomechanism of adhesion, colonization, invasion and transmission [8,9]. *S. aureus* isolates from patients with osteomyelitis, endocarditis, septic arthritis and bacteraemia associated with infected intravenous catheters demonstrate higher collagen binding ability than isolates from patients with uncomplicated bacteraemia or from coagulase-negative staphylococci infected individuals [10]. *S. aureus* associated with toxic shock syndrome displays a unique ability to interact with type I and type II collagens [11].

Potential risk of nosocomial transmission has been increasing among postoperative illnesses due to healthy nasal carriage of pathogenic staphylococci among hospital personnel [12]. Staphylococci colonizing in the vestibulum nasi of healthy carriers remain in close alliance with mucin overlaying the cilia in the airways. Mucins are known to inhibit epithelial cell binding or invasion of various bacterial pathogens, through specific or non-specific interactions [13,14]. The aim of the present investigation is to elucidate the effects of mucin–staphylococci interaction on the binding of fibronectin (Fn), laminin (Lm) and type I and type IV collagens (Cn) to *S. aureus*, and, further, to examine the differences in mucin inhibitory profiles between *S. aureus* isolates from healthy nasal carriage and strains associated with different clinical infections.

Materials and methods

Bacterial strains

A total of 89 *S. aureus* strains isolated from 35 patients suffering from diverse clinical manifestations and 54 human individuals with asymptomatic nasal carriage were studied. Laboratory reference strains *S. aureus* Cowan 1 and *Micrococcus lysodeikticus* were used as positive and negative controls during the serum and connective tissue protein binding assays. Blood agar grown fresh isolates were inoculated into trypticase soy broth (Difco, Detroit, MI, U.S.A.) and incubated at 37°C for 18 h with constant shaking. Bacterial cells were harvested after centrifugation at 400 *g*. Cells were washed once in 0.15 M phosphate buffered saline, pH 7.2 (PBS), and the density was adjusted to 10^{10} bacteria/ml photometrically at 540 nm.

Chemicals

Mucin purified from bovine submaxillary glands was purchased from U.S. Biochemicals Corp., Cleveland, OH, U.S.A. Fibronectin was purified from human plasma on gelatin Sepharose according to Veunto & Vaheri [15]. Vitrogen-100, a Cn preparation containing 95% type I and 5% type III Cn, was purchased from Collagen Corp., Palo Alto, CA, U.S.A. Type II collagen was from Southern Biotechnology Associates Inc., Birmingham, AL, U.S.A. Type IV Cn and Lm, both purified from basement membrane of the Engelbreth-Holm-Swarm transplantable mouse tumour, were purchased from Collaborative Research Inc., Bedford, MA, U.S.A. All chemicals used for the preparation of buffer solutions were of analytical grade.

[^{125}I]-labelled protein binding assay

Fn, Lm and type I and type IV Cn were labelled with Na [^{125}I] (specific activity 3.5 mBq/µl; Amersham, U.K.) using Iodobeads (Pierce Chemicals Co., Rockford, IL, U.S.A.) [16]. Binding assays were performed as described earlier by Naidu *et al.* [11]; briefly, bacteria (approximately 10^9 cells) in 100 µl PBS were incubated for 1 h in polystyrene tubes, with an equal volume of [^{125}I]-labelled respective protein (in PBS containing 0.1% of ovalbumin) corresponding to a radioactivity measurement of 2–2.5 × 10^4 cpm at 37°C for 1 h. After adding 2 ml ice-cold PBS (containing 0.05% azide and 0.1% Tween 20), tubes were centrifuged at 4500 *g* for 15 min and the supernatant was carefully aspirated. Bound radioactivity in the

bacterial pellet was measured, using a gamma counter (LKB Wallac Clingamma 1271, Turku, Finland). Residual radioactivity from incubation mixtures containing no bacteria (500–600 cpm) was considered as background and subtracted from the values obtained from incubations containing bacteria. Samples were always tested in duplicate.

Inhibition/displacement studies with unlabelled mucin

Unlabelled mucin preparations (100 µg in 100 µl volume diluted in PBS) were added to respective [^{125}I]-labelled serum or connective tissue protein (in 100 µl volume) and mixed with 10^9 bacterial cells (in 100 µl volume). Parallel preparations without unlabelled mucin served as controls.

One nasal carriage isolate, strain Nig-6, with optimum mucin inhibitory profiles for serum and connective tissue protein binding was selected for experiments on concentration (mucin) dependent displacement of labelled serum and connective tissue proteins. Increasing amounts (in the range of 0.001–10 mg) of unlabelled mucin (in 100 µl volumes diluted in PBS) were mixed with [^{125}I]-labelled proteins (in 100 µl volumes) and the experiment was performed essentially as described above. Both of the preparations (final volume of 300 µl) were tested for [^{125}I]-labelled protein binding as described above.

Results

S. aureus isolates from nasal carriage and clinical sources were tested for binding to [^{125}I]-labelled Fn, type-I, type-II and type-IV Cn and Lm (Table 1). Nasal carriage isolates showed a higher binding to all of the proteins tested, compared with strains isolated from different clinical materials. Among both groups of isolates laminin binding was pronounced. All three types of collagens showed similar binding profiles between individual groups of isolates.

Mucin interaction studies were performed with selected strains, comprising a total of 20 nasal carriage and 10 clinical isolates, positive for serum and connective tissue protein binding (Fig. 1). The mean binding of nasal carriage isolates for Fn (15.8%), Cn-I (26.8%), Cn-IV (15.3%) and Lm (22.8%) declined significantly following a prior incubation with 100 µg of mucin, reaching 9.6% (Fn), 14.8% (Cn-I), 8.9% (Cn-IV) and 12.9% (Lm). However, under similar conditions the mean binding of clinical isolates for Fn (12.3%), Cn-I (13.1%), Cn-IV (9.0%) and Lm (10.8%), after mucin incubation showed no marked variation, i.e. Fn (10.7%), Cn-I (12.0%), Cn-IV (8.1%) and Lm (9.6%).

The cumulative inhibitory effect of mucin on serum and connective tissue protein binding of both groups of *S. aureus* isolates is shown in Fig. 2. Nasal carriage isolates demonstrated a three-fold higher mucin mediated inhibition profile to [^{125}I]-labelled serum and connective

Table 1. [^{125}I]-labelled serum and connective tissue protein binding to *Staphylococcus aureus* strains isolated from healthy nasal carriage and clinical sources

	Positive for [^{125}I]-protein binding					
	Nasal carriage		Clinical source		Cumulative	
	No.	%	No.	%	No.	%
Fibronectin	24	(44.4)	12	(34.3)	36	(40.5)
Type I collagen	22	(40.7)	10	(28.6)	32	(35.9)
Type II collagen	21	(38.9)	8	(22.9)	29	(32.6)
Type IV collagen	21	(38.9)	9	(25.7)	30	(33.7)
Laminin	34	(63.0)	15	(42.9)	49	(55.1)

A total of 89 strains comprising 54 nasal carriage and 35 clinical isolates were tested for serum and connective tissue protein binding as described in Materials and methods.

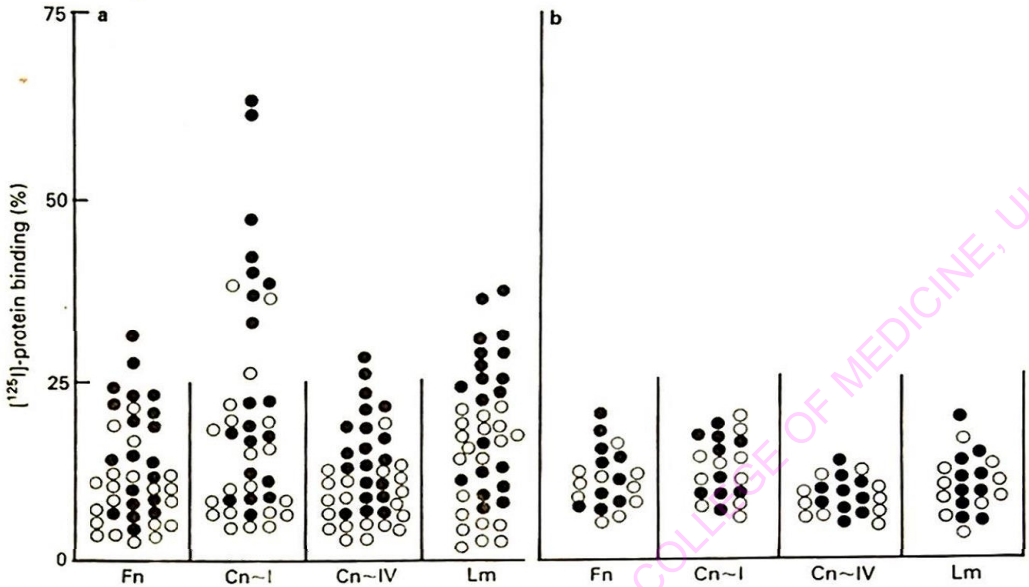

Fig. 1. Binding of [^{125}I]-labelled serum and connective tissue proteins to *S. aureus* from (a) nasal carriage and (b) clinical isolates in the presence (○) and absence (●) of mucin.

tissue protein binding (Fn 39.4%, Cn-I 44.7%, Cn-IV 42.0% and Lm 43.5%) than clinical isolates (Fn 13.3%, Cn-I 8.0%, Cn-IV 9.8% and Lm 11.2%).

One nasal carriage isolate, *S. aureus* strain Nig-6 was selected and further investigated in competitive displacement studies with unlabelled mucin (Fig. 3). [^{125}I]-labelled proteins and increasing amounts of unlabelled mucin (in the range 0.01–100 mg/ml) competed for binding. A concentration (mucin) dependent decrease in binding with all of the [^{125}I]-labelled proteins was observed. Mucin concentrations of 100, 150, 175 and 250 μg/ml elicited a 50% displacement of [^{125}I]-labelled Lm, Cn-I, Cn-IV and Fn uptake, respectively, when incubated with 10^9 bacterial cells.

Discussion

Serum and connective tissue protein binding has been argued as an anchoring mechanism for pathogenic bacteria in host tissue adhesion and colonization [8,9]. In the present study, *S. aureus* strains associated with healthy nasal carriage commonly demonstrated a higher serum and connective tissue binding than strains isolated from clinical manifestations. Interestingly, strains belonging to nasal carriage group also showed a significant degree of inhibition in binding to serum and connective tissue proteins after incubation with mucin. Mucin concentration dependent inhibition of protein binding also suggests that specific receptors for lectin-type interactions are probably present on nasal carriage isolates.

Ramphal & Pyle have demonstrated that adherence of *Pseudomonas aeruginosa* to tracheal epithelium was inhibited by rat tracheal mucin binding to a specific sialic acid receptor on bacterial cells [6]. Similarly, soluble salivary mucus glycoprotein has been shown to competitively inhibit the binding of streptococci to buccal epithelial cells [17]. Guinea pig colonic mucus has been reported to inhibit the HeLa cell invasion by *Shigella flexineri* [18].

Miedzobrodzki *et al.* [19] have shown the inhibitory effect of skimmed milk on fibronectin and collagen binding to bovine mastitis strains of staphylococci and coagulase-negative staphylococci. Skimmed milk has also been shown to reduce the surface hydrophobicity and cell surface charge of staphylococci [20]. These

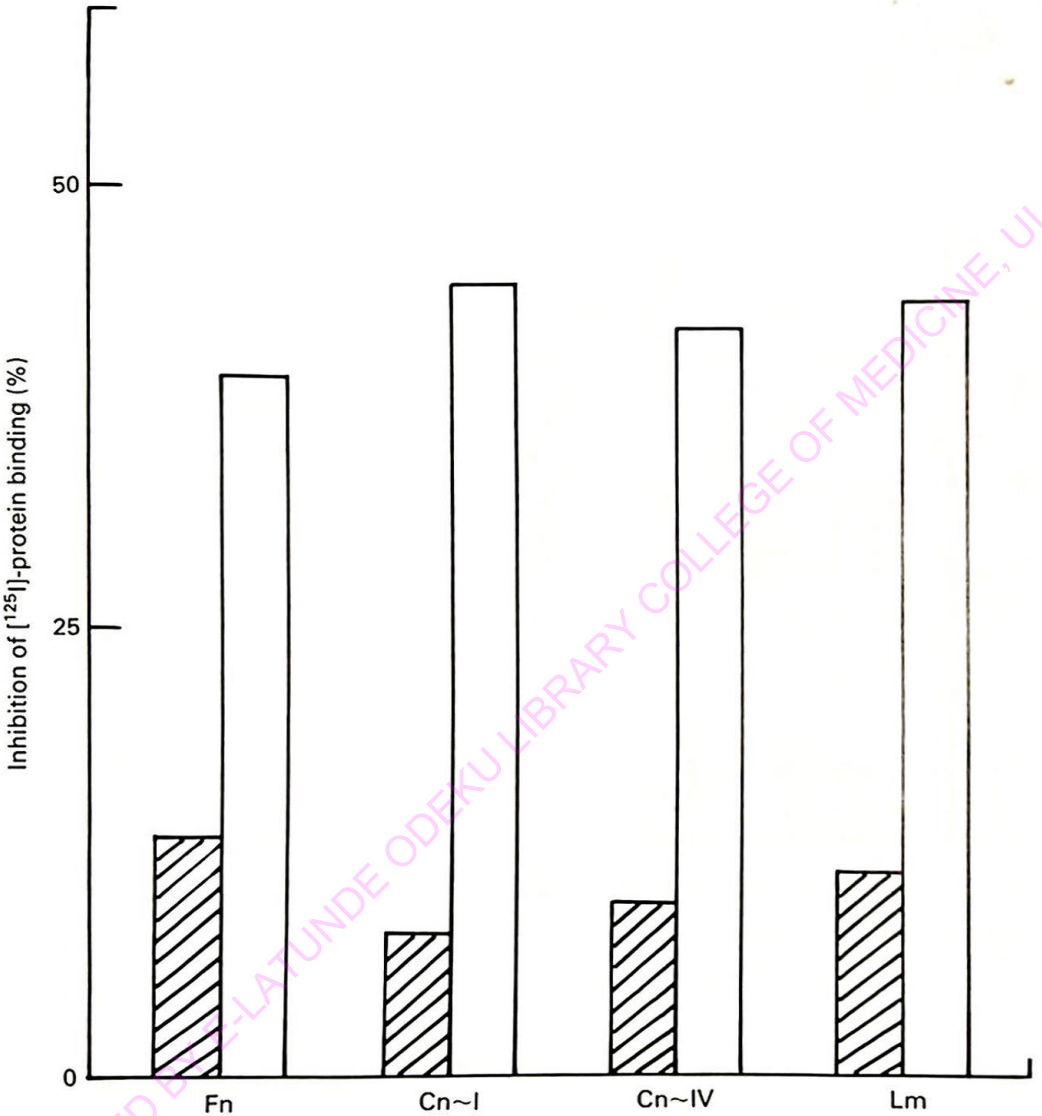

Fig. 2. Effect of mucin (cumulative inhibition profile) on serum and connective tissue protein binding to nasal carriage (□) and clinical (▨) isolates of *S. aureus*: Fn = fibronectin; Cn-I = type I collagen; Cn-IV = type IV collagen; Lm = laminin.

findings suggest the importance of mucosal secretory compounds (as in milk) in the inhibition of bacterial binding to epithelial and tissue matrix proteins.

Mucins are present on almost all mucosal surfaces with a role attributed in the host defence, such as protecting the underlying surfaces from bacterial adhesion. Malfunction of mucus has been suggested as an important factor in the development of ulcerative colitis, peptic ulceration and cystic fibrosis [21–23]. The carbohydrates found in the glycosidyl side chains of mucin may also provide potential binding sites for luminal proteins and organisms

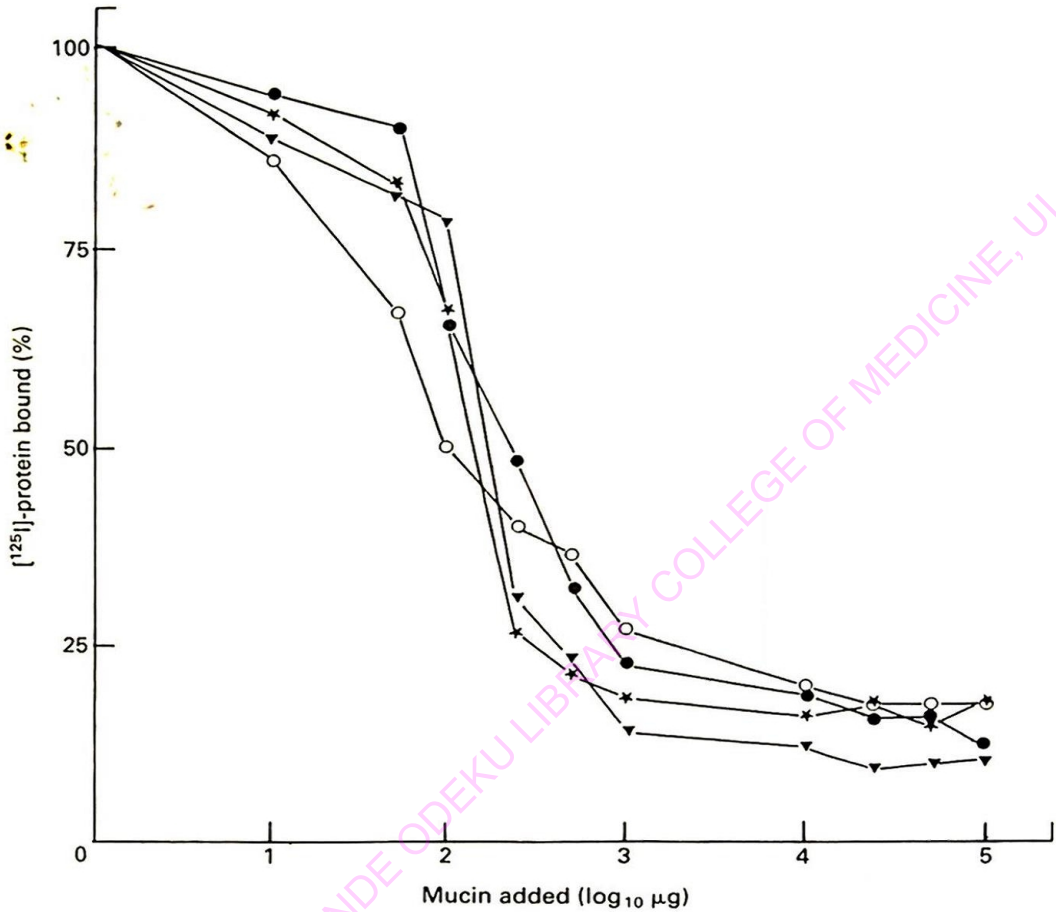

Fig. 3. Mucin concentration dependent displacement of [^{125}I]-labelled serum and connective tissue protein uptake by *S. aureus* strain Nig-6 (a nasal carriage isolate): ● = fibronectin; ★ = type I collagen; ▼ = type IV collagen; ○ = laminin.

for lectin type of interactions [24]. The glycosidyl residues of mucin cross-link with other glycoproteins [1], however, in our *in vitro* experiments mucin did not cause co-operative binding and no increase was observed in serum and connective tissue uptake by staphylococcal cells. Mucin or lectin type of interactions are not well-documented with staphylococcal pathogens. However, specific haemagglutinins have been recently reported on the cell surface of staphylococci [25].

Finally, clinical isolates of *S. aureus* showed low or no interaction with mucin. The biological role of this difference in mucin mediated inhibition of serum and connective tissue protein binding between nasal carriage and clinical isolates of *S. aureus* is not known.

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(*Accepted* 5 *March* 1990)