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Binding of Cloned S-Fimbriated E. coli to Human Buccal Epithelial Cells – Different Inhibition of Binding by Neonatal Saliva and Adult Saliva[a,b]

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Summary

Investigations were carried out on the adhesion of cloned S-fimbriated *E. coli*, labelled with fluoresceinisothiocyanate (FITC) to human buccal epithelial cells. Fluorescence microscopic analysis revealed binding of bacteria to 75–95% of epithelial cells. Inhibition experiments with fetuin, α_1-acid glycoprotein and N-acetyl neuraminic acid confirmed the specificity of bacterial binding to sialoglycoproteins. Further studies using saliva as an inhibitor resulted in a 4–5 times stronger binding inhibition by newborn saliva in comparison to adult saliva coinciding with a 4–5 times higher content of total N-acetyl neuraminic acid in samples of newborn saliva. In Western blot analysis sialoglycoprotein bands with a molecular weight >200 kD reacting with wheat germ agglutinin (WGA), were only identified in samples of newborn saliva. These bands are classified as mucins on account of molecular weight and staining. These data suggest that saliva mucins could represent a major defense mechanism against bacterial infections at a stage of ontogeny where the secretory IgA-system is not yet developed.

Zusammenfassung

Die Adhäsion clonierter, Fluoresceinisothiocyanat (FITC)-markierter, S-Fimbrien tragender *E. coli* an menschliche Mundschleimhautzellen wurde untersucht. Die fluoreszenzmikroskopische Auswertung ergab, daß 75–95% der Schleimhautzellen Bakterien gebunden hatten. Die Spezifität der Bindung der Bakterien an Sialoglykoproteine konnte durch Inhibitionsexperimente mit Fetuin, saurem α_1-Glykoprotein und N-acetyl-Neuraminsäure

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bestätigt werden. Wurde als Inhibitor Speichel eingesetzt, so ergab sich für den Speichel Neugeborener eine 4–5fach stärkere Inhibition als für Erwachsenenspeichel. Parallel dazu ergab die Untersuchung der Speichelproben für Neugeborene einen 4–5fachen höheren Gehalt an Gesamt-N-acetyl-Neuraminsäure. In Westernblot Analysen konnten nur in Proben nativen Speichels Neugeborener mit Wheat Germ Agglutinin (WGA) reagierende Sialoglykoproteinbanden mit Molekülmassen >200 kD identifiziert werden, die aufgrund ihres Molekulargewichtes und Färbeverhaltens der Klasse der Mucine zuzuordnen sind. Speichelmucine können einen wichtigen Abwehrmechanismus gegen Infektionen in einer Periode der kindlichen Entwicklung darstellen, in der das sekretorische IgA-System noch nicht voll entwickelt ist.

Introduction

Capsule-type K1 and S-fimbriae are common features in *E. coli* strains causing neonatal sepsis and meningitis (5). As the adhesion of pathogenic microorganisms to mucosal surfaces is a prerequisite for infection of the organisms (1), the adhesion of S-fimbriated *E. coli* to human buccal epithelial cell was investigated in the present study. S-fimbriae bind to sialyl-(α 2–3) galactoside structures (7), frequently part of mucinous sialoglycoproteins.

Material and Methods

Bacteria. Adhesion experiments were performed using *E. coli* strain HB 101 (pANN 801–4) which expresses S-fimbriae encoded by a recombinant plasmid (4). Bacteria were adjusted to 10^{10} bact/ml, labelled with 100μ FITC (Sigma Chemicals Co., St. Louis MO, USA) and subsequently washed several times.

Buccal epithelial cells. Buccal epithelial cells were obtained from healthy adult non-smokers by scraping buccal mucosa with a spoon-shaped spatula several times. Cells were adjusted to 1×10^5 cells/ml in PBS.

Collection of whole saliva. Saliva of healthy 1–5 day old newborns was collected 6–7 h after feeding (human milk or infant formula) by careful continous suction using a thin sterile plastic catheter. Saliva of healthy 20–30 year old adults was obtained in the same manner.

Determination of sialic acid. To determine the total sialic acid content, 100 μl of saliva were hydrolysed and then analysed according to the thiobarbituric acid method of *Warren* (9) using N-acetyl neuraminic acid (NANA) as standard.

Preincubation of bacteria with inhibitor. 125 μl of labelled bacteria suspension adjusted to 2×10^8 bact/ml were mixed with 125 μl of the potential inhibitor of adhesion (saliva in different volumina) for 15 min under constant shaking in an ice bath. The following inhibitors and concentrations were used: Human glycophorin (1 mg/ml), α_1-acid glycophorin (10 mg/ml), fetuin (5 mg/ml), asialo human glycophorin (1 and 8 mg/ml), NANA (1–20 mg/ml, adjusted to physiological pH), newborn saliva (20, 50, 100 μl), adult saliva (100, 200, 300 μl). Neuraminidase treated newborn and adult saliva (100 μl), periodate (pH 4, 3) treated newborn and adult saliva (100 μl). All chemicals were obtained from Sigma.

Binding of bacteria to buccal epithelial cells. Bacteria or bacteria plus inhibitor mixture were added to the epithelial cell suspension (ratio cells to bacteria 1 : 1000) and incubated for 60 min in an ice bath under gentle shaking. In control experiments epithelial cells were treated with *V. cholerae* neuraminidase (Behring, Marburg, FRG). The number of attached bacteria was analysed by fluorescence microscopy. 50 epithelial cells were evaluated for each experiment.

Electrophoresis and Western-blotting. After sodium dodecyl sulfate polyacrylamide gel electrophoresis of saliva samples (10 μl) gels were fixed and stained with Coomassie blue

(CB) and periodic acid-Schiff reagent (PAS). Electroblots onto nitrocellulose were stained with peroxidase conjugated wheat germ agglutinin (WGA).

Results

After incubation with FITC-labelled S-fimbriated *E. coli*, 75–95% of buccal epithelial cells from 10 different donors had bound bacteria when evaluated fluorescence-microscopically. Human glycophorin, fetuin and α_1-acid glycoprotein inhibited binding to markedly different degrees (s. Table 1). NANA, as a monovalent control, was unable to inhibit binding, asialo human glycophorin, as a polyvalent control substance, was hardly able to inhibit. Pretreatment of epithelial cells with neuraminidase reduced the number of cells capable of binding bacteria to 27%. Newborn saliva inhibited bacterial adhesion 4–5 times more than adult saliva (Table 2). 50 µl newborn saliva caused approximately the same degree of inhibition as 200 µl adult saliva, coinciding with a 4–5 times higher content of N-acetyl neuraminic acid in newborn saliva samples compared with adult samples. Periodate treated saliva adjusted to pH 4.3 (leading selectively to neuraminic acid residue oxidation) as well as neuraminidase treated saliva reduced adhesion inhibition considerably. Gel electrophoresis of saliva samples stained with CB (Fig. not shown) showed a band >200 kD that decolorised positively when stained with PAS (Fig. not shown) and reacted with WGA in Western-blotting (Fig. 1).

Discussion

In the present study, S-fimbriated *E. coli*, important pathogenic organisms causing sepsis and meningitis in the neonatal period (5), are shown to be capable of specific adhesion to sialoglycoproteins of human buccal epithelial cells. Newborn saliva inhibits adhesion 4–5 times more than adult saliva. This could be a microbiological correlate for the finding of *Schmidt* et al. (8), confirmed by our own investigations, that newborn saliva contains a considerably higher amount of neuraminic acid in comparison to adult saliva. The ability of newborn saliva to inhibit adhesion of pathogenic microorganisms is most probably an important defence mechanism in the neonatal

Table 1. Inhibition of bacterial adhesion to human buccal epithelial cells

Inhibitor[a]	[mg/ml]	Inhibition of adhesion (%)
α_1-acid glycoprotein	10	43 (± 11)[b]
Fetuin	5	42 (± 7)
Human glycophorin	1	42 (± 5)
Asialo human glycophorin	1	9 (± 4)
Asialo human glycophorin	8	28 (± 6)
NANA	20	0

[a] Inhibitor concentrations varied between 1 to 20 mg/ml. Experiments with inhibition close to 50% are presented. If 50% could not be reached, individual values are given.
[b] Standard deviations of mean values are given in brackets.

Table 2. Inhibition of bacterial adhesion by saliva of newborns and adults

Inhibitor	NANA µg/ml	µl/ml bact. susp.	Inhibition of adhesion (%)
Saliva of newborns	233 (± 38)[a]	20	17 (± 10)
n = 5		50	44 (± 7)
		100	61 (± 9)
Saliva of adults	56 (± 33)	100	9 (± 6)
n = 5		200	46 (± 12)
		300	57 (± 15)
Saliva of newborns			
Neuraminidase treated	217	100	30
Periodate treated	217	100	21
n = 2			
Saliva of adults			
Neuraminidase treated	57	100	4
Periodate treated	57	100	1
n = 2			

[a] Standard deviations of mean values are given in brackets.

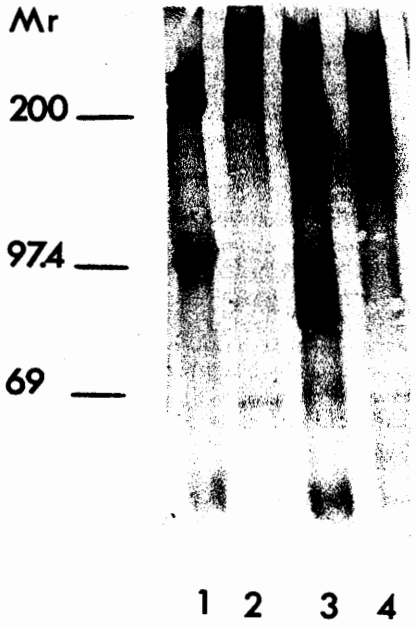

Fig. 1. Western blot analysis of saliva samples. Western blots of sliva were stained with peroxidase conjugated WGA. Protein markers used are indicated by bars and molecular mass (lane 1),
adult saliva (lane 2),
newborn saliva (lane 3 and 4).

period, when the secretory IgA-system is not yet fully developed (2). Further studies should be performed to determine whether the sialoglycoproteins, classified as mucins because of their molecular weight and staining, present only in newborn saliva are responsible for the adhesion preventing effect of newborn saliva. However, the anti-infectious potential of mucins, which express a multitude of receptor-analogue structures for bacterial adhesins and thus prevent bacterial adhesion, has been documented in recent studies (3, 6).

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