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S-Fimbriae Mediated Adhesion of *Escherichia coli* to Human Buccal Epithelial Cells is Age Independent

Summary: S-fimbriated *Escherichia coli*, which cause sepsis and meningitis in the newborn, bind to sialic acid-containing glycoprotein structures on the surface of human buccal epithelial cells. The dependence of this binding on host age was examined. S-fimbriated *E. coli* adhered in comparable numbers to cells in newborns, infants, children and adults (23.0 ± 8.6; 23.1 ± 11.5; 24.7 ± 7.9; 28.9 ± 8.8). Thus, the increased susceptibility of neonates to infections caused by S-fimbriated *E. coli* cannot be explained by enhanced adhesion to epithelial cells.

Zusammenfassung: *Die S-Fimbrien vermittelte Adhäsion von Escherichia coli an menschliche Mundschleimhautzellen ist altersunabhängig.* S-Fimbrien tragende *Escherichia coli*, die Sepsis und Meningitis im Neugeborenenalter verursachen, binden an sialinsäurehaltige Glycoproteine auf der Oberfläche menschlicher Mundschleimhautzellen. Wir untersuchten die Abhängigkeit der Bindung vom Alter des Schleimhautzellenspenders. S-Fimbrien tragende *E. coli* banden in vergleichbarer Zahl an Zellen von Neugeborenen, Säuglingen, älteren Kindern und Erwachsenen (23,0 ± 8,6; 23,1 ± 11,5; 24,7 ± 7,9; 28,9 ± 8,8). Die vermehrte Empfänglichkeit von Neugeborenen für Infektionen, die durch S- Fimbrien tragende *E. coli* verursacht werden, kann nicht mit einer verstärkten Adhäsion an Mundschleimhautzellen erklärt werden.

Introduction

Escherichia coli is the most frequently identified pathogen in neonates with gram-negative bacterial sepsis and meningitis. In contrast, it is rarely responsible for these infections in older children [1,2]. Before invasive infections become manifest, bacteria must adhere to epithelial cell surfaces in a first pathogenetic step [3]. In gram-negative bacteria the adhesion is usually mediated by fimbriae [4–6]. One of these is termed S-fimbriae because it is specific for sialic acid-containing glycoproteins on mucosal cell surfaces [7,8]. Besides K1 capsular polysaccharide antigen, S-fimbriae have been shown to be associated with virulence in *E. coli* causing sepsis and meningitis [9–11]. We have recently shown that cloned S-fimbriated *E. coli* bind to sialic acid-containing structures on human buccal epithelial cells [12].

In the present study we investigated whether age-dependent development of adherence to buccal epithelial cells could, at least in part, explain the rare occurrence of invasive *E. coli* infections beyond the neonatal period.

Materials and Methods

Bacteria: Adhesion experiments were carried out with *E. coli* strain HB101 (pANN 801-4), which encodes S-fimbriae [13]. In a few experiments strain IH 3084 (kindly provided by Dr. *Korhonen*, University of Helsinki, Finland), isolated from a newborn infant with meningitis and carrying S-fimbriae, was used; however, expression of S-fimbriae in this strain was unstable *in vitro* so that we were unable to carry out reproducible experiments. Cloned bacteria were grown on NB agar supplemented with 0.1% glucose and tetracycline (30 mg/l) for 18 h at 37°C. They were harvested with ice-cold 20 mM sodium borate buffer pH 9.0 and washed twice with the same buffer. Following adjustment to 10^{10} bacteria/ml, 1 ml of the suspension was labelled with 100 µg fluorescein isothiocyanate (FITC, Sigma Chemical Co., St. Louis, Mo., USA) according to the method described by *Parkkinen* et al. [14].

Isolation of buccal epithelial cells: Buccal epithelial cells were obtained from healthy individuals of different ages by scraping buccal mucosa with a spoon-shaped spatula several times. After washing three times, cells were microscopically adjusted to $1x10^5$ cells/ml in PBS.

The following age groups, each including at least ten subjects, were examined: 0 – 28 days, 29 days – 12 months, 13 months – 5 years, 6 – 10 years, 11 – 17 years (n = 10) and 18 – 30 years (n = 15).

Binding of bacteria to buccal epithelial cells and preincubation with inhibitor: FITC-labelled bacteria were incubated with the epithelial cell suspension as described previously [12]. In order to confirm the specificity of the bacteria/cell interaction, aliquots of epithelia from each age group were incubated with a mixture of bacteria plus 1 mg/ml human glycophorin (Sigma), an established potent inhibitor of S-fimbriae mediated adhesion [12].

We did not perform kinetic studies or studies comparing different inhibitors to each other, nor was it our aim to investigate the intra-individual day-to- day variation of adhesion [15]. The number of bacteria bound to cells was analyzed by fluorescence microscopy. Fifty epithelial cells were analyzed for each experiment. All experiments were performed in duplicate. The arithmetical means of the age groups were compared by analysis of variance. The power calculation is based on a comparison of the age groups 0 – 28 days and 18 – 30 years.

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Table 1: Binding of S-fimbriated *Escherichia coli* to human buccal epithelial cells from individuals at different ages.

Age of epithelial cell donors	No. of bacteria bound/cell	
	Mean	(95% confidence interval)
0–28d (n = 10)	23.0	(17.5–28.3)
29d–12m (n = 10)	23.1	(16.0–30.2)
13m–5y (n = 10)	24.7	(19.8–29.6)
6y–10y (n = 10)	24.0	(17.5–30.6)
11y–17y (n = 10)	27.1	(20.3–34.0)
18y–30y (n = 15)	28.9	(24.5–33.4)

d = days, m = months, y = years.

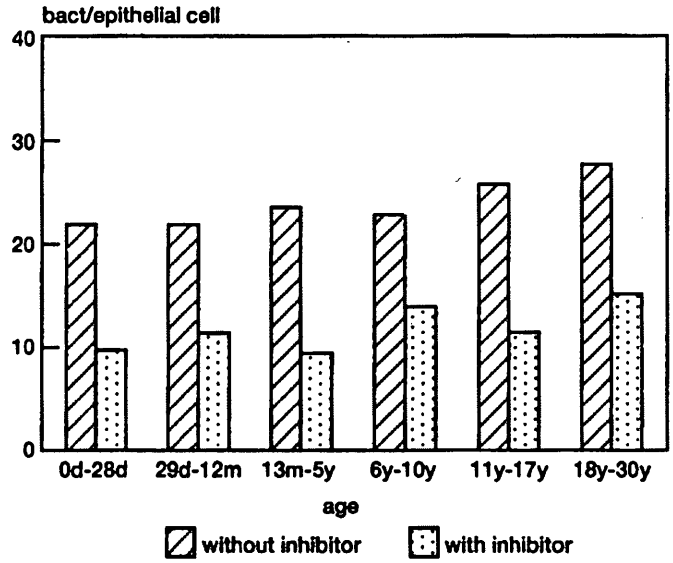

Figure 1: Binding of S-fimbriated *Escherichia coli* to human buccal epithelial cells from individuals at different ages, with or without preincubation of bacteria with inhibitor (human glycophorin, 1 mg/ml). Each bar represents the mean of ten epithelial cell donors, except the two bars for the 18–30 year age group, which represent the mean of 15 epithelial cell donors. d = days, m = months, y = years.

Results

Fluorescence microscopy of buccal epithelial cells after incubation with FITC-labelled S-fimbriated *E. coli* showed that 75–95% of cells had bound the bacteria. Binding characteristics of the clinical isolate IH 3084 did not differ from those of the cloned strain HB 101 (pANN 801-4) (data not shown). The mean numbers of adhering bacteria per cell in each age group were not significantly different ($p > 0.1$) (see Table 1). Given the sample size of ten in each group, any difference between group means of at least 15 bacteria per cell would have been detected with a probability of 80%. Preincubation of bacteria with human glycophorin markedly reduced the binding of bacteria, confirming the specificity of binding via neuraminic acid-containing glycoproteins (Figure 1).

Discussion

Neonatal septicaemia and meningitis still represent major problems in pediatric infectious diseases [16]. Sepsis and meningitis caused by *E. coli* are associated with a mortality of up to 31%. Of surviving infants, 29% develop neurological sequelae [9,17]. Buccal epithelial cells and cells of the oropharynx are targets for bacterial adhesion and represent an entry route for *E. coli* causing sepsis and meningitis in the newborn period [18]. Intestinal colonization with *E. coli* K1 strains followed by bacteremia in an infant rat model was found to be age-related [19]. *Clegg* et al. [20] examined the effect of host age on the adherence of mannose-sensitive fimbriated *E. coli* K1 to human oral epithelial cells using a sample size smaller than ours. They found no association between age and epithelial cell adherence. *Schaeffer* et al. found that adhesion of uropathogenic *E. coli* to epithelial cells was greater in adult females with frequent urinary tract infections than in healthy controls [15,21]. However, *Clegg* et al. found no association between the health status of children in their study group and the adhesion of type-I fimbriated *E. coli* to epithelial cells. Therefore, we have now investigated the possible association between age and adhesion of S-fimbriated *E. coli* to epithelial cells in healthy cell donors only, although it is difficult to include large numbers of newborn subjects in such studies. In a recent study we were able to demonstrate adhesion of S-fimbriated *E. coli* to human buccal epithelial cells [12]. Our results suggest that age-dependent differences in susceptibility to *E. coli* meningitis and sepsis cannot be explained by differences in adhesion of S-fimbriated *E. coli* to buccal epithelial cells. Other age-related host factors will have to be considered and analyzed. Neonatal mucosal surfaces could be more permeable or S-fimbriae could bind to specific binding sites occurring only in the neonatal brain [14].

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