

Bacterial Adherence to Human Buccal Epithelial Cells and Its Possible Role in Bacterial Colonization in Human Oral Cavity*

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The ability of several species of streptococcus and staphylococcus to adhere to human buccal epithelial cells was studied *in vitro* by using bacteria and epithelial cells isolated from human buccal cavity.

Viridans streptococci were found adhering in highest numbers (65 ± 8 bacteria per epithelial cell) to epithelial cells. *Streptococcus pyogenes* adhered in great numbers (44 ± 4), whereas *Streptococcus pneumoniae* (26 ± 2), *Staphylococcus aureus* (21 ± 2), *Staphylococcus epidermidis* (14 ± 2) adhered poorly. These data showed that bacteria differed in their ability to adhere to human buccal epithelial cells. This difference in adhesive ability between bacterial species may correlate with the ability of the bacteria to colonize oral surface of human.

Key Words: Bacterial adherence, Colonization.

The presence of certain indigenous and pathogenic bacteria is known to be limited to only some host species and only some tissues in the host. It has been suggested that the ability of the bacteria to adhere to epithelial surface plays an important role in the host and tissue tropism. Several studies have shown that the predilection of indigenous oral bacteria for colonizing oral surface is correlated with their ability to adhere to the oral epithelial cells *in vitro* (Gibbons and Houte, 1971; Gibbons

and Houte, 1975; Liljemark and Gibbons, 1971). Also the ability of pathogenic bacteria to adhere to epithelial cells has been reported for *Streptococcus pyogenes* (Ellen and Gibbons, 1972; Ellen and Gibbons, 1974; Selinger *et al.*, 1978), *Escherichia coli* (Eden *et al.*, 1977; Kallenius and Winberg, 1978; Schaeffer *et al.*, 1979), *Neisseria gonorrhoeae* (Ward and Watt, 1972), *Vibrio cholerae* (Jenes *et al.*, 1976; Jones and Freter, 1976), *Sbigella flexneri* (Izhar *et al.*, 1982), and gram negative bacilli (Johanson *et al.*, 1980; Johanson *et al.*, 1979). Thus, bacterial adherence to epithelial cells may be regarded as one of important determinants influencing colonization of indigenous and

Received June 18, 1982

* This study was supported by a grant (1980) from the Yuhan corporation, Seoul, Korea.

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pathogenic bacteria.

In the present study, the ability of several species of streptococcus and staphylococcus to adhere to human buccal epithelial cells was determined by using *in vitro* assay. And we discussed the possible role of bacterial adhesive ability in colonization in human oral cavity.

MATERIALS AND METHODS

Preparation of bacterial suspensions. Several species of bacteria, viridans streptococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, maintained in our department were used.

For each experiment, the bacteria were cultured overnight at 37°C in brain heart infusion (Difco) broth. After culture, the bacterial culture suspensions were vigorously shaken by Cyclo-mixer (Clay-Adams) for 5 min to disrupt long chains or clusters. Bacteria were collected by centrifugation at 2000xg for 10 min, washed three times with 0.01M phosphate in saline (PBS), pH 7.0. The bacteria were resuspended in PBS to yield approximately 1×10^8 bacteria per ml by McFarland turbidity standards.

Preparation of human buccal epithelial cells. Three healthy donors were followed throughout this experiment. Epithelial cells from the donors were obtained by buccal scrapings with a sterile cotton-tipped swab, and were pooled in PBS. The cells were centrifuged at 200xg for 10 min, washed three times with PBS. The buccal epithelial cells were resuspended in PBS to obtain a concentration of 1×10^5 cells per ml by counting in a hemocytometer.

***In vitro* bacterial adherence assay.** The ability of bacteria to adhere to human buccal epithelial cells was studied by *in vitro* system (Fig. 1) modified from the previously described methods (Alkan *et al.*, 1977; Ellen and Gibbons,

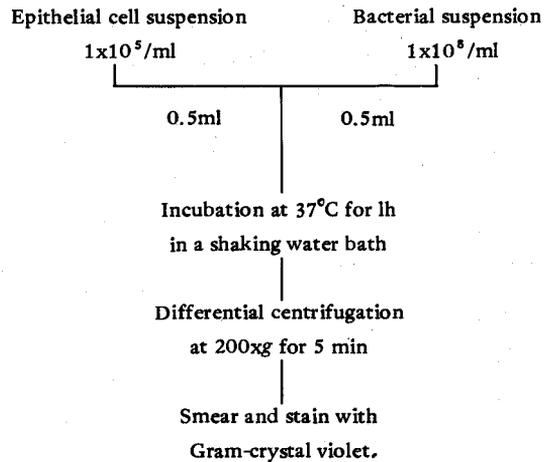


Fig. 1. *In vitro* bacterial adherence assay.

1972, Ellen and Gibbons, 1974; Gibbons *et al.*, 1976). In assay, 0.5ml each of epithelial cell and bacterial suspension were mixed and incubated at 37°C for 1hr with continuous shaking (170 oscillations/min) in a shaking water bath (Precision). For control, epithelial cell suspensions were incubated with PBS instead of bacterial suspension. After incubation, the mixed suspensions were washed to be free from unattached bacteria by repeated differential centrifugation at 200xg for 5 min. Direct smears were prepared from each epithelial cell suspension and stained with Gram-crystal violet. The number of bacteria attached per epithelial cell was determined by enumeration of 25 epithelial cells under the oil immersion lens of light microscope.

RESULTS

The ability of each bacterial species to adhere to human buccal epithelial cells is shown in Table 1. The average number of bacteria that had been already attached, at the time of collection, to control epithelial cells was 6. Different

Table 1. Adherence of several species of streptococcus and staphylococcus to the human buccal epithelial cells *in vitro*

Bacteria	Number of attached bacteria per epithelial cell
Viridans streptococci	65 ± 8*
<i>Streptococcus pyogenes</i>	44 ± 4
<i>Streptococcus pneumoniae</i>	26 ± 2
<i>Staphylococcus aureus</i>	21 ± 2
<i>Staphylococcus epidermidis</i>	14 ± 2
Control†	6 ± 1

* Mean and standard error

Mean value is based on data from three experiments.

† For control, epithelial cell suspensions were incubated with PBS.

species showed different abilities to adhere. Viridans streptococci were found adhering in highest numbers (65±8). *Streptococcus pyogenes* adhered in great numbers (44±4), whereas *Streptococcus pneumoniae* (26±2), *Staphylococcus aureus* (21±2), and *Staphylococcus epidermidis* (14±2) showed weak adherence.

DISCUSSION

The adherence of bacteria to epithelial cells is widely regarded as an important prerequisite for colonization and expression of virulence of the microorganisms (Beachey, 1981; Gibbons and Houte, 1975). The ability to adhere to specific tissues should be influenced by the characteristics of the bacterial and epithelial surfaces and the contents of the environments (Beachey, 1981; Gibbons *et al.*, 1976).

In the present investigation, we examined the ability of several species of streptococcus and staphylococcus to adhere to human buccal epithelial cells by using *in vitro* assay. Viridans streptococci, which constitute the major flora of human oral cavity, actually possess a distinct

ability to adhere to isolated human buccal epithelial cells, as noted previously by others (Gibbons and Houte, 1971; Gibbons and Houte, 1975; Gibbons *et al.*, 1976). In contrast, *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are normally not present in human oral cavity, exhibited weak ability to adhere. The relative differences in the ability of these organisms to adhere to isolated human buccal epithelial cells determined by *in vitro* assay correlated with their natural presence in human oral cavity. This correlation implies that the bacterial adherence is one of important determinants in colonization. *Streptococcus pyogenes* were found adhering in higher numbers than *Streptococcus pneumoniae* to human buccal epithelial cells. *Staphylococcus pyogenes*, which can infect pharynx under natural conditions were found to adhere well to pharyngeal and buccal epithelial cells *in vitro* (Ellen and Gibbons, 1974). The ability of *Streptococcus pyogenes* to adhere may correlate with the organism's virulence in pharynx (Ellen and Gibbons, 1972; Ellen and Gibbons, 1974; Selinger *et al.*, 1978). In contrast, *Streptococcus pneumoniae* adhered weakly to the buccal epithelial cells. The weak attachment of *Streptococcus pneumoniae* may correlate with the fact that these organisms are pathogens for lower respiratory tract of human. This suggests that the ability of bacteria to adhere to a given area may serve as one of virulence factors. This is further supported by other studies which showed several pathogenic bacteria display restricted range of host cells that they infect under natural conditions (Gibbons *et al.*, 1976; Hohmann and Wilson, 1975; Johanson *et al.*, 1980; Kallenius and Winberg, 1978; Selinger *et al.*, 1978).

Our data showed that each bacterial species differed in its ability to adhere to isolated human buccal epithelial cells *in vitro*. The results of this study suggest that the ability of bacteria

to adhere to isolated human buccal epithelial cells may play an important role in natural colonization and provide an opportunity for expression of virulence in human oral cavity.

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