INFECTION AND IMMUNITY, Sept. 1977, p. 546–549

Vol. 17, No. 3
Printed in U.S.A.

Bacterial Adherence to Nasal Mucosal Cells

R. ALY,[1,2]* H. I. SHINEFIELD,[2] W. G. STRAUSS,[1] AND H. I. MAIBACH[1]

Departments of Dermatology[1] and Microbiology,[2] University of California, San Francisco, California 94143

Received for publication 18 March 1977

The ability of several bacterial species to adhere to human nasal mucosal cells and their distribution on nasal mucosal surfaces was studied. *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes,* and *Pseudomonas aeruginosa* adhered to scraped nasal mucosal cells. In contrast, viridans streptococci and *Klebsiella pneumoniae* exhibited feeble or no adherence to nasal mucosal cells. *S. aureus* affinity for the nasal mucosal cells of carriers of *S. aureus* was greater than for those of the noncarriers ($P < 0.005$). Heat treatment of *S. aureus* did not block, but slightly reduced, its binding to mucosal cells. The data suggest a high degree of specificity involved in the adherence of bacteria to nasal mucosal cells. The greater affinity of *S. aureus* for the nasal mucosal cells of carriers (than noncarriers) seems to be a property of mucosal cells rather than bacteria.

Bacterial adherence to host cells has been documented for enteric (4, 12), cariogenic (8, 9), streptococcal (7, 12), and gonococcal bacteria (13). The bacteria vary in their ability to attach to epithelial cells (7). *Streptococcus pyogenes* possessing M antigen attach better to human epithelial cells than do avirulent mutants lacking this component (5). The pili of gonococci enable them to attach to epithelial cells (10, 13).

Whereas mechanisms of bacterial attachment to mucosal cells of the mouth, intestine, and vagina have been adequately studied, the process by which bacteria attach to nasal mucosal cells has not. Our goals in this investigation were (i) to develop methods by which bacterial adherence to nasal mucosal cells can be demonstrated and (ii) to delineate differences of the nasal mucosal cells between carriers and noncarriers of *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and preparation of mucosal cell suspension. Wooden tongue depressors, cut lengthwise, were used for collecting the mucosal cells from the anterior nares. Subjects gently scraped the mucosa of each anterior nare 10 times. The mucosal cells were collected in 0.075 M sodium phosphate buffer. The cell suspensions obtained from two to five subjects were pooled. The cells were rinsed and washed three times on a membrane filter (14-μm pore size; Millipore Corp.) with 0.075 M sodium phosphate buffer to dislodge secretion and unattached bacteria (6). The cells were counted in a hemocytometer and adjusted to concentrations of 10^4 to 10^5/ml.

Selection of nasal carrier and noncarriers of *S. aureus*. Subjects from whom a strain of *S. aureus* was recovered in at least three of four consecutive weekly cultures were designated as carriers; those from whom *S. aureus* was not recovered were termed noncarriers.

Preparation of bacterial suspension. Strains of bacteria, *S. aureus, Staphylococcus epidermidis, Streptococcus pyogenes,* viridans streptococci, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa,* maintained in our laboratory were utilized. The bacteria were grown in Trypticase soy broth (Difco), washed three times in phosphate buffer, and resuspended at about 10^8 organisms per ml for the adherence experiment.

Mixture of bacteria and mucosal cells. One milliliter each of mucosal cell preparation and bacterial suspensions were mixed and incubated at 35°C for 90 or more min. After incubation the cells were washed free of unattached bacteria in a 14-μm filter, using 40 ml of 0.075 M sodium phosphate buffer. Direct smears were prepared from each epithelial cell suspension and stained for 15 s with Gram-crystal violet. The number of bacteria attached to the epithelial cells was examined under the oil immersion lens of a light microscope. Control epithelial cell suspensions were incubated with buffer instead of bacteria.

Heat treatment of *S. aureus*. An overnight culture (18 h) of *S. aureus* was divided into equal portions. One sample was placed in the refrigerator and the other was placed in a 60°C water bath for 2 h to heat kill the bacteria. Each bacterial suspension was allowed to return to room temperature. One milliliter of bacterial suspension of each preparation was added to 1 ml of mucosal cell suspension. Heat-killed bacteria were subcultured on Trypticase soy agar plates to determine their viability.

RESULTS

Attachment of *S. aureus* to nasal mucosal cells. Nasal mucosal cells treated with *S. aureus* are shown in Fig. 1A. Mucosal cells incubated with phosphate buffer but not with bacte-

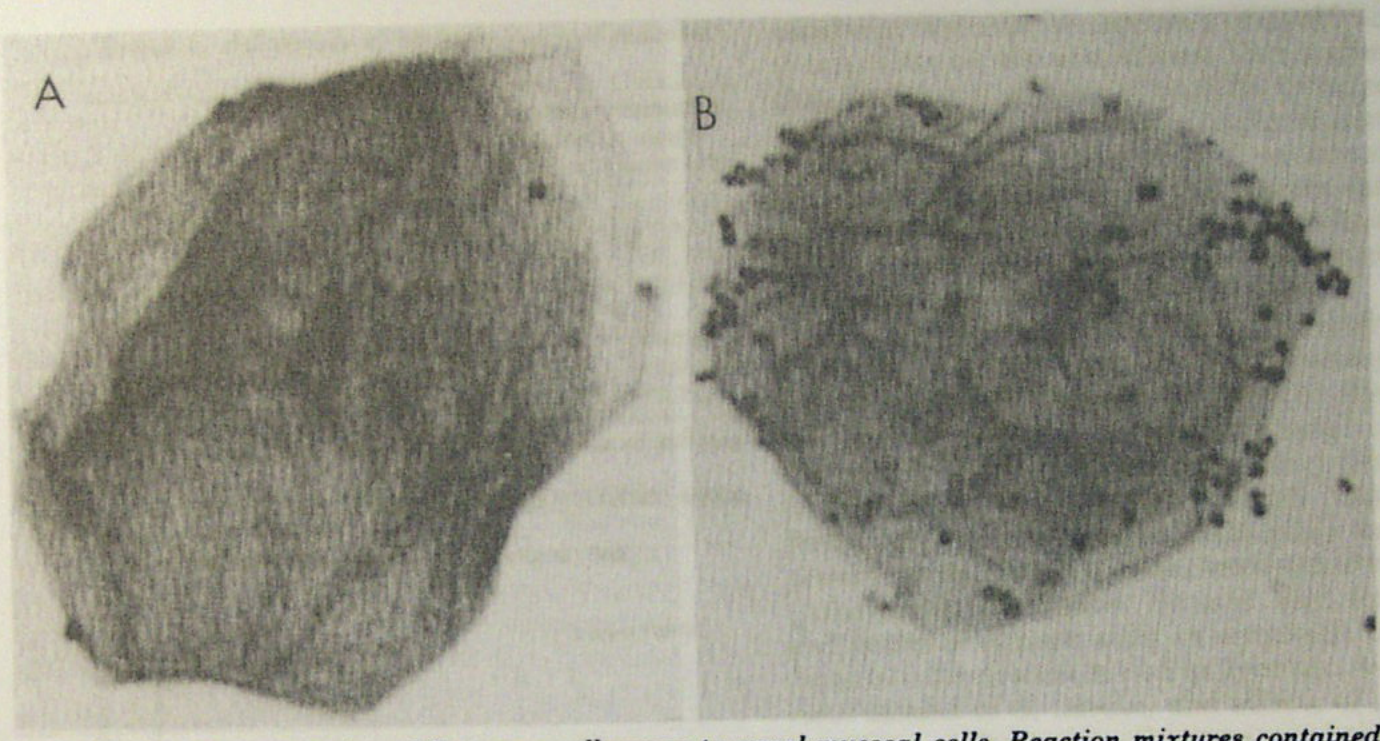

FIG. 1. *(A) Demonstration of S. aureus adherence to nasal mucosal cells. Reaction mixtures contained 10^8 staphylococci and 10^5 mucosal cells. (B) For controls, the mucosal cells were treated with phosphate buffer only.*

ria served as the control (Fig. 1B). The average bacterial count on the mucosal cells before washing with phosphate buffer was 14 (range, 4 to 19; 10 subjects).

Demonstration of selective ability of bacteria to nasal mucosal cells. The ability to attach to mucosal cells of several bacteria is shown in Table 1. *P. aeruginosa, S. epidermidis, S. aureus,* and *Streptococcus pyogenes* demonstrated significant adherence to nasal mucosal cells; viridans streptococci and *K. pneumoniae* showed little ability to attach to mucosal cells.

Demonstration of optimum time of *S. aureus* adherence to mucosal cells. Maximum adherence of *S. aureus* occurred between 90 and 120 min of incubation (Fig. 2). In other studies, epithelial cell-bacteria mixtures were incubated for 30 min (7).

Demonstration of *S. aureus* adherence to nasal mucosal cells of carriers and noncarriers of *S. aureus*. Mucosal cells from persistent carriers and noncarriers of *S. aureus* were collected (20 subjects), and their affinity for *S. aureus* adherence was determined. The adherence of *S. aureus* to the nasal mucosal cells for carriers was significantly greater ($P < 0.005$) than that from the noncarriers, i.e., 132 ± 82 for carriers and 67 ± 70 for noncarriers (20 subjects in each group).

Adherence of heat-killed *S. aureus* to nasal mucosal cells was determined. Heat killing of bacteria did not completely block the binding of *S. aureus* to mucosal cells; however, a small quantitative reduction in their attachment was noted (Table 2).

DISCUSSION

For certain bacteria, particularly *S. aureus*, the nose is the primary site of multiplication and dissemination. By depressing nasal staphylococci, a rapid reduction of skin and aerial staphylococci occurs (14). With the present in vitro model utilizing isolated nasal mucosal cells and bacteria, the host-parasite relationship can be closely studied. We developed methods by which bacterial adherence to nasal cells could be demonstrated. A selective ability of bacteria to attach to nasal mucosal cells was noted. When test bacteria and nasal mucosal cells were experimentally mixed, significant adherence occurred with *P. aeruginosa, S. epidermidis, S. aureus, Streptococcus pyogenes,* and diphtheroids, but not with viridans streptococci and *K. pneumoniae.*

The feeble attachment of viridans streptococci was of considerable interest; this may correlate with the fact that their presence in the anterior nares is seldom noted (2, 9, 11). *Streptococcus pyogenes* and *P. aeruginosa* showed good attachment despite the fact that these are not seen in a healthy adult population (3, 11). Other host and ecological factors may be operative in vivo and may have been altered in our in vitro model.

These data indicate that there is a high degree of specificity involved in the adherence of bacteria to nasal mucosal cells. Staphylococci

TABLE 1. *Adherence of bacteria to nasal mucosal cells*

Bacteria[a]	Avg bacterial count/cell	Avg background count/cell[b]	Statistical significance (P)[c]
S. aureus (10)	53 ± 24	5 ± 2	<0.0001
S. epidermidis (5)	58 ± 16	3 ± 2	<0.0001
Viridans streptococci (6)	3 ± 1	3 ± 1	<0.7387
Streptococcus pyogenes (5)	120 ± 85	3 ± 2	<0.0001
P. aeruginosa (6)	153 ± 92	2 ± 1	<0.0001
K. pneumoniae (6)	4 ± 3	2 ± 1	<0.0064
Diphtheroids	47 ± 41	2 ± 1	<0.0001

[a] The number in parentheses is the number of subjects used.

[b] The mucosal cells were treated with phosphate buffer instead of test bacteria.

[c] Significance was determined by standard t test.

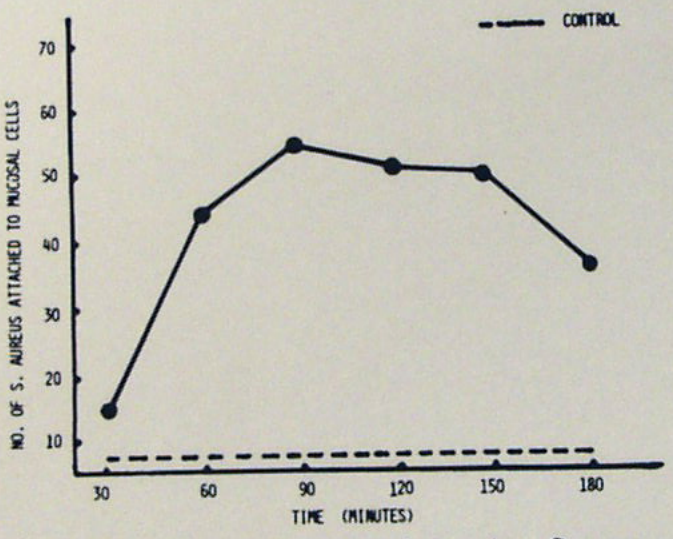

FIG. 2. *Determination of optimum time. S. aureus adherence to nasal mucosal cells.*

TABLE 2. *Adherence of heat-killed and viable S. aureus to nasal mucosal cells*

Treatment[a]	Avg counts
Heat-killed	33
Viable	38
Control	2.5

[a] Bacteria were killed in a water bath at 60°C for 2 h. The controls were treated with phosphate buffer only. The difference between heat-killed and viable bacteria was not statistically significant.

that constitute the major flora of the anterior nares (2) possess a distinct advantage over viridans streptococci. Viridans streptococci constitute the major aerobic flora of the mouth.

The question as to why some people become nasal carriers of *S. aureus* and others do not, though often asked, has never been answered. We previously explored genetic and microbial factors (1, 2). Adherence of *S. aureus* to the nasal mucosal cells for carriers was significantly greater than for the noncarriers. This suggested that the greater affinity of bacteria to mucosal cells of staphylococcal carriers might be a property of the mucosal cells or host environment rather than bacteria. With the present model several bacterial and host factors can be investigated to determine the staphylococcal carriage status between carriers and noncarriers.

This investigation provides a tool for studying independently and separately the physiological effect of host and bacterial products on the adherence of bacteria to nasal mucosal cells. Knowledge of mechanisms controlling the adherence of bacteria to nasal mucosal cells (an initial step of host-parasite interaction) should provide leads toward a better understanding of pathogenesis of disease. Several host factors and bacterial factors, such as enzymatic treatment of cells, protein A-rich and protein A-deficient strains of *S. aureus*, and effects of nasal secretions on bacterial adherence, are being investigated.

ACKNOWLEDGMENTS

The excellent technical assistance of Charlene Shirley is gratefully acknowledged.

This work was supported by Public Health Service research grant AI 10856-04 from National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Aly, R., H. I. Maibach, H. R. Shinefield, and A. Mandel.** 1974. *Staphylococcus aureus* carriage in twins. Am. J. Dis. Child. **127**:486–488.
2. **Aly, R., H. I. Maibach, W. G. Strauss, and H. R. Shinefield.** 1970. Effect of systemic antibiotic on nasal bacterial ecology in man. Appl. Microbiol. **20**:240–244.
3. **Aly, R., H. I. Maibach, W. G. Strauss, and H. R. Shinefield.** 1972. Survival of pathogenic microorganisms on human skin. J. Invest. Dermatol. **58**:205–210.
4. **Bertshinger, H. W., H. W. Moon, and S. C. Whipp.** 1972. Association of *Escherichia coli* with the small intestinal epithelium. I. Comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. Infect. Immun. **5**:595–605.
5. **Ellen, R. P., and R. J. Gibbons.** 1972. M protein associated adherence of *Streptococcus pyogenes* to epithelial surfaces: prerequisite for virulence. Infect. Immun. **5**:826–830.
6. **Gibbons, R. J.** 1972. Ecology and cariogenic potential of oral streptococci, p. 371–385. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.
7. **Gibbons, R. J., and J. Van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
8. **Gibbons, R. J., and J. Van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
9. **Hallman, F. A.** 1937. Pathogenic staphylococci in the anterior nares: their incidence and differentiation. Proc. Soc. Exp. Biol. Med. **36**:789–794.

10. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. 8:255–263.
11. **Rosenburg, J.** 1962. Microorganisms indigenous to man, p. 9. McGraw-Hill, New York.
12. **Savage, D. C.** 1972. Survival on mucosal epithelia, epithelial penetration and growth in tissues of pathogenic bacteria, p. 25–28. *In* H. Smith and J. H. Pearce (ed.), Microbial pathogenicity in man and animals. The University Press, Cambridge.
13. **Swanson, J.** 1973. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. 137:571–589.
14. **White, A., and J. Smith.** 1964. Nasal reservoir as the source of extranasal staphylococci, p. 679–683. Antimicrob. Agents Chemother. 1963.