In Vivo Localization of *Staphylococcus aureus* in Nasal Tissues of Healthy and Influenza A Virus-Infected Ferrets (42903)

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Abstract. **An *in vivo* ferret model was used to study the association of *Staphylococcus aureus* with specific tissues of the nasal cavity in both control and influenza A virus-infected animals. Ferrets were inoculated intranasally with various doses of influenza A3/Hong Kong/1/68 virus. On Days 2, 5, 9 and 14, four or five virus-inoculated and two uninoculated controls were challenged intranasally with a 1-ml volume of radiolabeled *S. aureus* (3 mg dry wt), a clinical isolate of low passage history. Ferrets were allowed to clear the staphylococci *in vivo* for 60 to 90 min before sacrifice. The animals were anesthetized, exsanguinated, and decapitated, and the lower jaw was removed. The nasal fossae were exposed by dissection and turbinates from the left nasal fossa were used for virus isolation. The median septum and tissues from the right nasal fossa, which included vestibule and anterior and posterior turbinates, were harvested and processed for radioassay. The percentage of recoverable staphylococci from virus-infected ferrets (Days 2 and 5) was ≥10-fold higher compared with controls and animals infected with suboptimal doses of virus; ≥76% of the recoverable staphylococci, whether from controls or virus-infected animals, was associated with the anterior turbinates. Histologic examination of the anterior turbinates from virus-infected ferrets, particularly on Days 2 and 5 postexposure to virus, showed that the staphylococci were adhering to desquamating respiratory epithelial cells. In contrast, the anterior turbinates from control ferrets uninoculated with virus and posterior turbinates from both control and virus-infected animals showed no evidence of bacteria adhering to host cells; instead, the staphylococci were found in association with the mucus gel layer of respiratory mucosa. Examination of vestibular tissue showed staphylococci in association with cells of the stratum granulosum in both virus-infected and control animals. Results of this study suggest that the early events of *S. aureus* interaction with different sites of ferret nasal tissues are effected by different mechanisms, and that the interaction is significantly enhanced by virus-infection.** [P.S.E.B.M. 1989, Vol 191]

Human influenza is predominantly an upper respiratory tract infection that is seldom lethal unless complicated by bacterial infection (1, 2). The mechanisms by which influenza promotes bacterial superinfection have been the subject of several recent reviews (3–6). During the past decade, our laboratory has been testing the hypothesis that influenza virus infection significantly enhances the adherence of specific bacterial pathogens that, in turn, may lead to colonization and superinfection. More recently, we have become interested in developing an animal model to test this hypothesis. Influenza virus was originally recovered in the ferret (7) and this animal remains the model of choice for studying the pathogenesis of influenza because the disease in ferrets closely resembles the clinical picture seen in humans (8). Although the ferret model has been used extensively in influenza research, it has been used in only three studies as a model of influenza-bacterial synergism (9–11). Brightman (9) demonstrated that ferrets experienced a more severe infection when challenged with a combination of influenza virus and group C streptococci than the mild infection that followed inoculation of virus alone or the absence of infection when inoculated with streptococci alone. Glover (10) showed that ferrets acquired group C streptococci via aerosol only if they were first infected with influenza A virus. The third and most recent study (11) was the first to investigate viral-bacterial synergism in the ferret from a mechanistic perspective. The study was based on data indicating that influenza A virus infection predisposes host cells to enhanced bacterial adherence *in vitro* (12–19). Control and influenza A

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virus-infected ferrets were sacrificed, the heads were harvested, and labeled bacteria were instillated intranasally. Results of the radioassay indicated that adherence of *Staphylococcus aureus* was significantly enhanced in virus-infected ferrets versus controls. These results led to this current study in which ferrets, uninfected, or virus-infected, were challenged intranasally with labeled *S. aureus* and were allowed a period of time to clear the bacteria prior to sacrifice. Subsequently, tissues from different sites were harvested from the nasal cavity and subjected to radioassay or histologic studies to determine the specific location(s) of staphylococci-host tissue interaction and to further define the nature of this interaction.

Materials and Methods

Virus. Influenza A/Hong Kong/1/68 virus (H3N2; ATCC VR-544; American Type Culture Collection, Rockville, MD) was propagated in the allantoic cavities of embryonated chicken eggs (20). The virus subtype was confirmed by hemagglutination inhibition (21) using antisera against the Hong Kong strain of virus kindly provided by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. Infectivity of allantoic fluids was also titrated in eggs and the EID_{50} was calculated according to the method of Reed and Muench (22). Stock virus was stored at −70°C until needed for ferret inoculation.

Radiolabeling of Staphylococci. *Staphylococcus aureus* strain (Ci-1) was used in this study and has been characterized previously (23). Staphylococci were labeled with [*methyl*-^{3}H]thymidine (15 Ci/mmol; ICN Chemical and Radioisotope Div., Irvine, CA) as described previously (24) and freeze-dried for storage. Immediately before a control or virus-infected ferret was challenged, 3.2 mg (dry wt) of labeled staphylococci were washed in Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, NY), pH 7.2, and suspended in a 1-ml volume of Hanks' balanced salt solution. A tuberculin syringe, with an attached 25-gauge ⅝-in needle, was used to vigorously disperse any clumps in the staphylococcal suspension and was also used to deliver drops of the suspension into the anterior nares.

Ferrets. Twenty-nine adult male ferrets, obtained from Triple-F (Fayre, PA), were processed and anesthetized as described previously (11). Animals were inoculated intranasally with a 1-ml volume of allantoic fluid containing either 2, 20, or 200 EID_{50} of virus. On Days 2, 5, 9, or 14 postexposure to virus, four animals were anesthetized and challenged with a 1-ml volume of ^{3}H-labeled staphylococci that was slowly applied intranasally, on the right side only, by allowing the animal to inspire one drop of the suspension at a time. Following inoculation, the animals were allowed to clear the bacteria for 60 to 90 min, after which time they were again anesthetized and then sacrificed by exsanguination via cardiac puncture. Blood specimens were allowed to clot and sera were collected for further testing. Each animal was decapitated, the lower jaw was removed, and the head was placed in a slotted box where it was sawed in half slightly lateral to the median sagittal plane, leaving the nasal septum undisturbed and exposing the tissues of the left nasal fossa. The following tissues were dissected from the right nasal fossa: median septum (Fig. 1A), vestibule, anterior turbinate, and posterior turbinate (Fig. 1B). The tissues were immediately processed for radioassay (three animals) or histopathology (one animal). The anterior turbinate was harvested from the left nasal fossa and placed into a 2-ml volume of HBSS containing 0.5% lactoalbumin hydrolysate (Difco Laboratories, Detroit, MI), and supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and gentamycin (50 μg/ml). Turbinate suspensions were stored at −70°C until used for virus isolation and quantitation in the plaque assay. Control animals were not inoculated with virus but were challenged with ^{3}H-labeled staphylococci and processed for radioassay or histopathology as described. One additional control animal received neither virus nor bacteria and was processed for histopathology only.

Radioassay. Each tissue specimen was transferred to a vial containing a 3-ml volume of 0.125% protease (type VI obtained from *Streptomyces griseus*; Sigma, St. Louis, MO) in sterile phosphate-buffered saline (FTA hemagglutination buffer, pH 7.2; Baltimore Biological Laboratory, Cockeysville, MD) and incubated at 37°C for at least 45 min with intermittent agitation. The vial was vortexed and the supernatant fluid, containing host cells, labeled staphylococci, and debris, was transferred to a test tube. The vial was rinsed with a 3-ml volume of sterile, deionized H_2O which was then added to the supernatant fluid. Cells were pelleted by centrifugation, the supernatant fluid was discarded, and the pellet was suspended in a 0.5-ml volume of H_2O. A 10-ml volume of ScintiVerse I scintillation cocktail (Fisher Scientific, Fair Lawn, NJ) was added to the suspended pellet, and radioactivity was measured in a Tracor Analytic model 6895 liquid scintillation spectrometer. The disintegrations per minute were converted to nanograms of staphylococci as previously described (11).

Histopathology. Immediately after harvesting, each tissue specimen was placed in a Uni-Cassette System (Miles Laboratories, Inc., Naperville, IL) and fixed in 10% neutral buffered formalin (American Scientific Products, McGaw Park, IL) for 24 to 48 h. Processing of specimens was performed in the Histopathology Laboratory, Department of Pathology, The University of Texas Health Science Center, San Antonio, TX. Briefly, each specimen was decalcified in TBD-2 (Shandon, Sewickley, PA), embedded in paraffin, and multiple sections (3-μm in thickness) were made in a stepwise fashion throughout the paraffin block. Sections

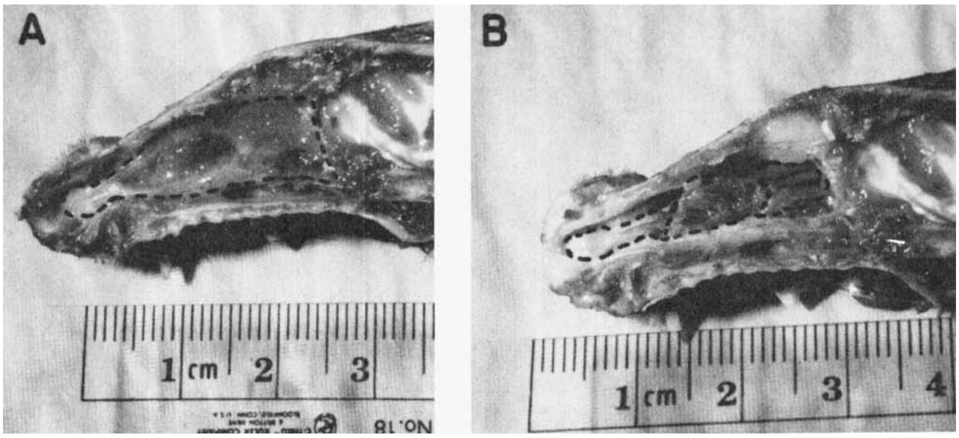

Figure 1. Median sagittal view of a ferret head after removal of the lower jaw before (A) and after (B) the median septum was removed. The outlined septum (A) was cartilaginous ventrally and bony dorsally. Removal of the septum exposed the right nasal fossa (B). Three areas are outlined: (i) the vestibule, beginning at the external nares, included a fibromuscular strand that attached dorsally to the turbinate; (ii) the anterior and middle turbinates were fan-like scrolls that appeared injected and glistening 48 hr after intranasal instillation of influenza A virus; and (iii) the posterior turbinate consisted of parallel finger-like scrolls.

were stained by hematoxylin and eosin, the Brown and Hopps (B&H) method for detection of gram-positive and gram-negative bacteria, and the periodic acid-Schiff method for visualization of mucopolysaccharides. Photomicrographs were made using Kodak Tmax 100 film.

Serology. Kaolin-treated sera were titrated by hemagglutination inhibition for antibodies against the infecting strain of influenza A virus (21).

Plaque Assay. After thawing, the suspensions of turbinates were vigorously vortexed. Madin-Darby canine kidney cells (NBL-2; American Type Culture Collection) were inoculated with 10-fold dilutions of supernatant fluids from the tissue suspensions and virus was allowed to grow for 3 to 5 days (34°C in a 5% CO_2 atmosphere) under an agar overlay medium containing trypsin according to the procedure described by Tobita *et al.* (25). Monolayers were fixed by adding Cornoy's solution (1 part glacial acetic acid and 3 parts 95% ethanol in water) to the agar overlay which was removed before staining with 1% crystal violet in 50% ethanol. Plaques were counted and the total number of plaque-forming units was determined for each specimen.

Results

Figure 2 shows the mean amount of *S. aureus* associated with the four tissue specimens harvested from the nasal fossae of virus-infected and control ferrets. For control animals, only 0.3% of the total amount of labeled staphylococci, introduced into the right nasal fossa, was recoverable in these tissues 60–90 min after inoculation, and 99% of the recoverable staphylococci were associated with the anterior turbinate. Results obtained from animals inoculated with 2 (not shown) or 20 EID_{50} of virus (Fig. 2) did not differ significantly from that of control animals, and only 0.4% of the staphylococci was recovered. All animals inoculated with these two doses of virus were positive for virus isolation with a mean number of 5.25×10^{12} plaque-forming units in anterior turbinates harvested on Day 2, the only time period tested postexposure to

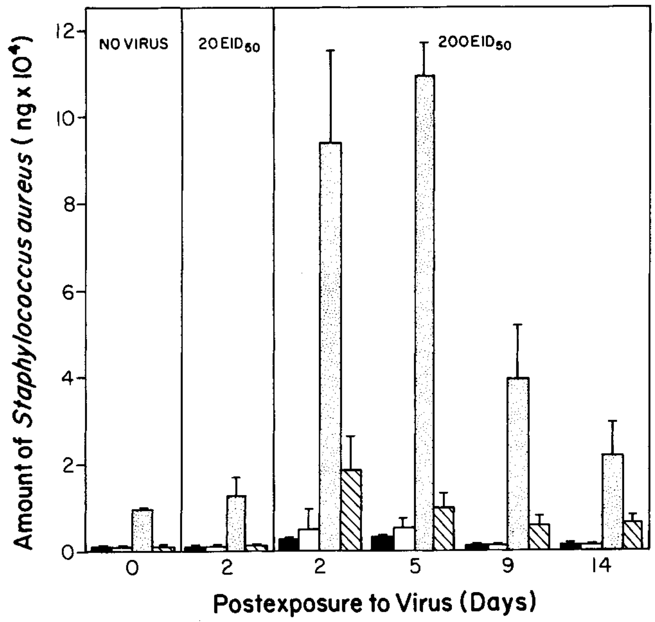

Figure 2. Mean association of ^{3}H-labeled *Staphylococcus aureus* with the median septum (closed bar), vestibular tissue (open bar), anterior turbinate (stippled bar), and posterior turbinate (striped bar) from the right nasal fossae of ferrets that were not inoculated with virus or were inoculated with either 20 EID_{50} or 200 EID_{50} of influenza A3/Hong Kong/1/68 virus. Thin bars represent the standard deviation of results.

virus. In contrast, tissues from ferrets inoculated with the highest test dose of influenza A virus (200 EID_{50}) showed significantly increased association with labeled staphylococci, as seen in Figure 2. The percentage of recoverable staphylococci was 3.4 (Day 2), 3.9 (Day 5), 1.0 (Day 9), and 0.9 (Day 14); of the total recoverable staphylococci, 78% (Day 2), 86% (Days 5 and 9), and 76% (Day 14) were associated with anterior turbinate. Over time, the mean number of influenza A virus plaque-forming units, isolated from anterior turbinates, decreased from 6.8×10^5 (Day 2) to 1×10^4 (Day 5); no virus was isolated from tissues harvested on Days 9 and 14. As virus titers decreased, antibody against the infecting virus increased with time from a mean titer of 80 (Day 2) to 320 (Day 5) and 10,240 (Days 9 and 14).

Although quantitative data accumulated from the radioassays indicated the level of association of staphylococci with specific tissues in the nasal cavity, the nature of the association was only revealed by a histologic examination of the tissues before and after virus infection. In both virus-infected and control animals, bacteria and host cell interaction were seen in the tissue from the vestibule (Fig. 3); numerous cocci appeared to associate with cells of the stratum granulosum which were in the process of keratinization. In contrast, nonkeratinized stratified squamous epithelium that formed the ventral attachment of the anterior turbinate and fully keratinized stratum corneum showed no adhering staphylococci.

Another pattern emerged for the anterior turbinate; staphylococcal adherence was markedly different between control and virus-infected animals. Figure 4 shows the normal architecture of tissue from a control animal in which regular ciliated columnar epithelium, interspersed with goblet cells, lined the air passages. These ciliated cells were overlaid by mucus gel which was loaded with staphylococci. Bacteria were seen only in association with mucus; no bacteria-host cell interaction was observed even in areas where the epithelium appeared to be mucus free. By Day 2, postexposure to virus, broad areas of the turbinate showed desquamation of the ciliated columnar epithelium and goblet cells, leaving only a basal layer lining the air passage. The air passages contained exudate comprised primarily of desquamated epithelial cells and segmented neutrophils (Fig. 5). In areas where staphylococci were observed, they appeared only in association with desquamated cells or cells in the process of desquamation (Figs. 5 and 6). The remaining basal layer was bacteria free. Similar results were obtained from tissue harvested on Day 5. By Day 9, tissue repair was in an advanced stage with evidence of transitional stratified squamous and stratified columnar epithelium, which in some areas appeared to be ciliated. Bacteria were more difficult to find, but when present appeared to be associated with desquamated cells, segmented neutrophils, and strands of mucus gel. On Day 14, the last time period tested, the epithelium was mostly stratified ciliated columnar cells and staphylococci were associated primarily with strands of mucus gel.

In the normal animal, the posterior turbinate was composed of olfactory epithelium, which had a layer of superficial ciliated columnar cells. This layer was overlaid by a mucus gel that contained staphylococci. Even after virus infection, the bacteria were still associated with mucus gel (Fig. 7); no staphylococci-host cell interaction was observed. We were not able to visualize staphylococci associated with septum from control or virus-infected animals; histologically, no mucus gel layer was apparent and, grossly, the mucosal

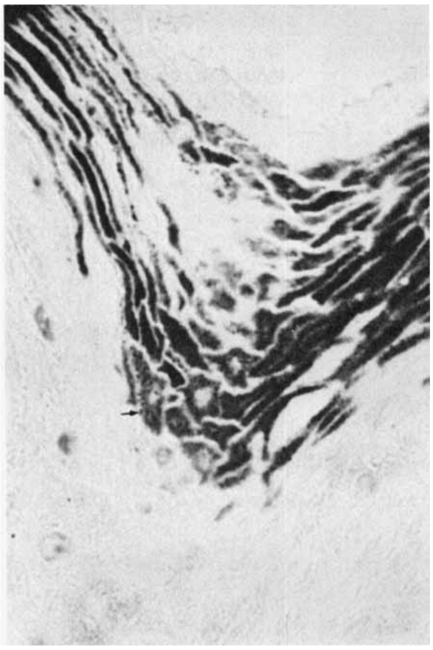

Figure 3. Oblique section of the vestibular tissue taken from a normal ferret challenged with *S. aureus* demonstrated that cells of the stratum granulosum were loaded with adherent gram-positive staphylococci (arrow) (B & H, original magnification ×500).

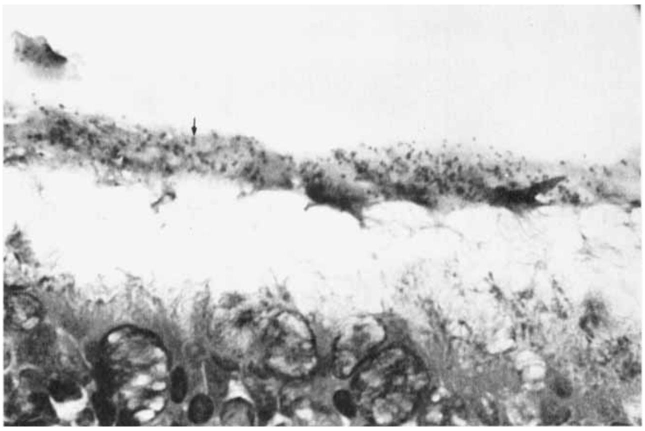

Figure 4. Oblique section of the anterior turbinate from a normal ferret challenged with *S. aureus*. Cilia of the columnar epithelium was covered by a layer of mucus gel that was loaded with gram-positive staphylococci (arrow). Air passages were free of exudate (B & H, original magnificaiton ×500).

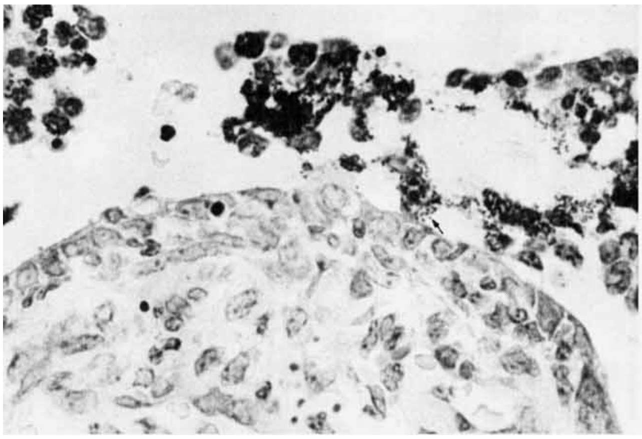

Figure 5. Anterior turbinate from a ferret on Day 2 postexposure to influenza A virus and challenged with *S. aureus*. The ciliated columnar epithelium and goblet cells have been desquamated exposing a basal layer of cells. Adherent staphylococci (arrow) were seen associated with desquamated cells and cells that appeared to be in the process of desquamation (B & H, original magnification ×500).

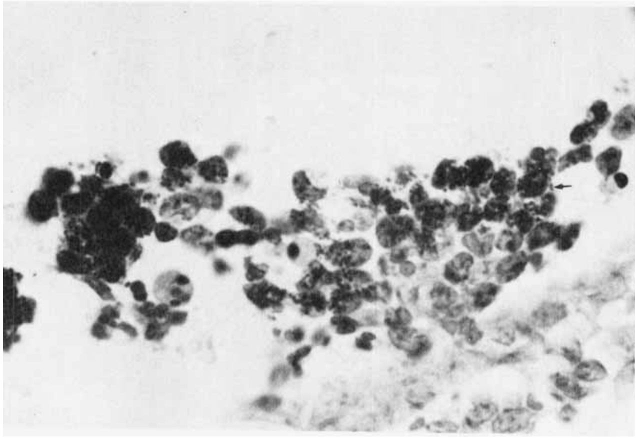

Figure 6. Anterior turbinate from a ferret on Day 2 postexposure to influenza A virus and challenged with *S. aureus*. Staphylococci (arrow) were seen with respiratory epithelial cells that were being desquamated from the mucosal surface (B & H, original magnification ×500).

epithelium was only loosely attached to cartilage and bone so it is possible that the organisms were simply lost in processing of specimens.

Discussion

We have previously used a ferret model radioassay system to compare the amount of ^{3}H-labeled *S. aureus* remaining in the nasal cavity and oropharynx of control and influenza A virus-infected animals that were first sacrificed, then instilled intranasally with staphylococci (11). In the present study, we modified the assay system in two ways: (i) labeled staphylococci were inoculated *in vivo* and the ferrets were allowed to clear the organisms before being sacrificed and (ii) specific tissues were harvested directly from the nasal fossa and tested for the presence of radiolabeled staphylococci without any wash steps. From 76 to 99% of the recoverable staphylococci were associated with the anterior turbinates of both virus-infected and control animals (Fig. 2). The amount of staphylococci recovered from virus-infected ferrets was significantly higher when compared with controls, particularly if the virus-infected tissue was harvested 2–5 days after virus inoculation. It is important to note that the mere presence of virus in the turbinates of ferrets exposed to low concentrations of virus inoculum was not sufficient to enhance staphylococcal association above that of uninfected control tissue (Fig. 2), which suggests that virus infection initiates a process that must occur before enhanced association is detectable. This observation is further supported by the fact that, even in animals infected with an optimal dose of virus, enhancement does not occur sooner than a full 48 hr after virus inoculation (data not shown).

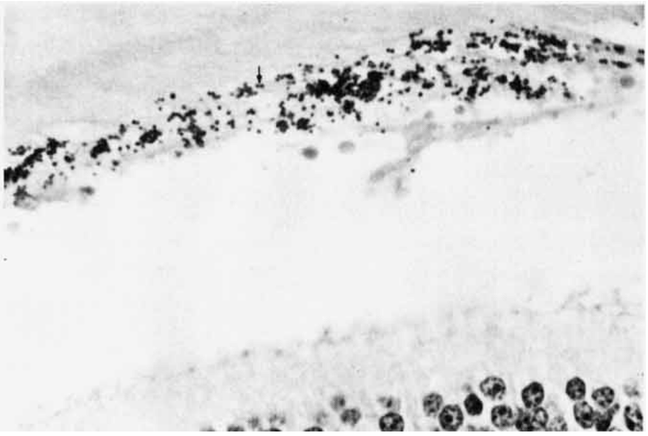

Figure 7. Posterior turbinate from a ferret on Day 5 postexposure to influenza A virus and challenged with *S. aureus*. Staphylococci (arrow) were associated with nonstructured mucinous exudate in the air passage located above the olfactory epithelium (B & H, original magnification ×500).

The radioassay is an objective and quantitative system that enables direct comparison of data between different animals and between tissues of the same animal; however, this assay does not permit direct observation of the interaction between staphylococci and tissue. For this reason, we examined the various tissues from control and virus-infected animals microscopically. It was, in fact, this aspect of the study that revealed at least three mechanisms of staphylococci-host tissue interaction. In the first, staphylococci appeared to associate directly with cells undergoing keratinization in the stratum granulosum of vestibular tissue from both control and virus-infected ferrets. These results are similar to those reported by Bibel *et al.* (26) in which they noted increased *S. aureus* adherence to "high granular" cells collected by scraping the anterior nares of human subjects. At least one of the staphylococcal adhesins mediating attachment to human nasal mucosal cells appears to be teichoic acid (27, 28). Whether this same cell wall component also mediates adherence to the ferret vestibular cells is unknown.

The second *in vivo* mechanism of staphylococcal association did not involve bacterial-host cell interaction, but rather interaction of staphylococci with mucus gel. This type of interaction was clearly demonstrable by histologic examination of the anterior turbinate from a control ferret. As Freter (29) has so aptly pointed out, mucus gel may simply trap bacteria preparatory to removal or the gel may actually contain receptor substances capable of reacting with bacterial adhesins and thereby promote mucosal association by retaining bacteria in the gel. An example of the former case can be seen in a report by Parsons and Mulholland (30) in which they described mucin as an "anti-adherence factor" in protecting the bladder mucosa of rabbits against adherence by *S. aureus*. In contrast, Verghese *et al.* (31) used mucin as a virulence-enhancing factor by suspending *S. aureus* in mucin before endotracheal inoculation of control and emphysematous hamsters; in emphysematous hamsters, this combination resulted in impaired clearance and decreased survival. In these two reports, the association of staphylococci with mucin could represent either a nonspecific or specific interaction. Whether respiratory mucus gel does, in fact, contain specific receptors for *S. aureus*, as has been described for *Pseudomonas aeruginosa* (32) and *Bordetella bronchiseptica* (33), is unknown and merits further study.

The development and repair of nasal lesions in our animals followed the same time sequence and exhibited the same histopathology as described in detail in an excellent study by Francis and Stuart-Harris (34) on the nasal histology of normal and influenza A virus-infected ferrets. Influenza virus has been shown to preferentially infect the anterior turbinates of ferrets (7), so it seemed reasonable to assume that if staphylococcal adherence was enhanced by virus infection the phenomenon would most readily be detected in turbinates. Data from radioassays indirectly supported this assumption. Histologically, we found that by Day 2 postexposure to virus the respiratory mucosa of the anterior turbinate was in an advanced stage of desquamation, and it was at this time that we observed the third mechanism of staphylococcal association. Adherence-positive staphylococci were found in association with patches of mucosal cells that appeared to be in the process of desquamation or were already desquamated and present in the air passages. Liu (35) has shown that influenza virus specifically infects the ciliated columnar epithelial cells of the anterior turbinate and appears to spread between groups (patches) of these cells throughout the respiratory epithelium; infection leads, in turn, to desquamation and desquamated cells in the air passage remain positive for virus antigen by immunofluorescence. Whether staphylococcal adherence to these probable virus-infected epithelial cells is mediated by the same adhesin(s)-receptor(s) interaction described for our *in vitro* cell culture system (24, 36) remains to be determined. Histologically, the bacteria-host cell association appeared to be highest on Day 2 but was still observable on Day 5; by Day 14 regeneration of the respiratory mucosa was advanced and staphylococci appeared only in association with strands of mucus gel. Extensive adherence over the entire surface of the exposed basal layer was not observed on Days 2 or 5, even though the mucosa had lost its "protective" coat of mucus gel. Except for a previous report from our laboratory (11), no animal model has been used before to study viral-bacterial synergism in tissues of the nasal cavity (upper respiratory tract). However, the mouse model has been used by several investigators to examine the effect of influenza virus infection on bacterial adherence in the trachea (lower respiratory tract). Ramphal *et al.* (14) demonstrated that adherence of *P. aeruginosa* was enhanced, but only on Days 2 and 3 postexposure to virus; pseudomonads were found adhering to desquamating or desquamated mucosal cells only. Nugent and Pesanti (37) reported that *S. aureus* persisted longer in the tracheas of mice on Day 7 postexposure to virus than in uninfected controls. Recently, Plotkowski *et al.* (38) were successful in demonstrating significantly enhanced adherence of *Streptococcus pneumoniae* on Day 6 postexposure to virus but not on Days 2 and 4. Electron micrographs revealed that the pneumococci were attached to the fibrous network of the extracellular matrix of the exposed basement membrane and to a lesser extent to basal cells of the mucosal surface. These results strongly suggest a different mechanism of pneumococcal association in virus-infected mice from that seen with pseudomonads in infected mice and staphylococci in infected ferrets.

We have shown that association of *S. aureus* with the mucosa of the upper respiratory tract differs quantitatively and qualitatively between influenza virus-infected and control ferrets. In virus-infected animals, the amount of staphylococci remaining after a period of clearance is significantly increased compared with controls. Importantly, the staphylococci appear to bind directly to virus-infected respiratory epithelial cells in contrast to the mucous binding seen in control animals. Whether this cell-cell interaction and impaired clearance in the nasal cavity lead eventually to lower tract disease remains to be determined.

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1. Smith H, Sweet C. Pathogenesis of influenza virus infection in ferrets, a model for human influenza. In: Stuart-Harris CH, Potter CW, Eds. The Molecular Virology and Epidemiology of Influenza. New York: Academic Press, pp175–194, 1984.
2. Douglas RG. Influenza in man. In: Kilbourne RD, Ed. The Influenza Viruses and Influenza. New York: Academic Press pp395–481, 1975.
3. Jakab GJ. Viral-bacterial interactions in pulmonary infection. Adv Vet Sci Comp Med **26**:155–171, 1982.
4. Mills EL. Viral infections predisposing to bacterial infections. Ann Rev Med **35**:469–479, 1984.
5. Degre M. Interaction between viral and bacterial infections in the respiratory tract. Scand J Infect Dis **49**(suppl):140–145, 1986.

6. Babiuk LA, Lawman MJP, Ohmann HB. Viral-bacterial synergistic interaction in respiratory disease. Adv Virus Res **35**:219–249, 1988.
7. Smith W, Manch MD, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. Lancet **2**:66–68, 1933.
8. Sweet C, Smith H. Pathogenicity of influenza virus. Microbiol Rev **44**:303–330, 1980.
9. Brightman IJ. Streptococcus infection occurring in ferrets inoculated with human influenza virus. Yale J Biol Med **8**:127–134, 1935.
10. Glover RE. Spread of infection from the respiratory tract of the ferret. II. Association of influenza A virus and streptococcus group C. Brit J Exp Pathol **22**:98–107, 1941.
11. Sanford BA, Ramsay MA. Bacterial adherence to the upper respiratory tract of ferrets infected with influenza A virus. Proc Soc Exp Biol Med **181**:104–111, 1987.
12. Sanford BA, Shelokov A, Ramsay MA. Bacterial adherence to virus-infected cells: A cell culture model of bacterial superinfection. J Infect Dis **137**:176–181, 1978.
13. Fainstein V, Musher DM, Cate TR. Bacterial adherence to pharyngeal cells during viral infection. J Infect Dis **141**:172–176, 1980.
14. Ramphal R, Small PM, Shands JW Jr., Fischlschweiger W, Small PA Jr. Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect Immun **27**:614–619, 1980.
15. Davison VE, Sanford BA. Adherence of *Staphylococcus aureus* to influenza A virus-infected MDCK cell cultures. Infect Immun **42**:118–126, 1981.
16. Selinger DS, Reed WP, McLaren LC. Model for studying bacterial adherence to epithelial cells infected with viruses. Infect Immun **32**:941–944, 1981.
17. Davison VE, Sanford BA. Factors influencing adherence of *Staphylococcus aureus* to influenza A virus-infected cell cultures. Infect Immun **37**:946–955, 1982.
18. Sanford BA, Davison VE, Ramsay MA. Fibrinogen-mediated adherence of group A *Streptococcus* to influenza A virus-infected cell cultures. Infect Immun **38**:513–520, 1982.
19. George RC, Broadbent DA, Drasar BS. The effect of influenza virus on the adherence of Haemophilus influenzae to human cells in tissue culture. Br J Exp Pathol **64**:655–659, 1983.
20. Swain RHA, Dodds TC. Clinical Virology. Baltimore: Williams & Wilkins, pp58–60, 1967.
21. Kucher RJ. Isolation and identification of animal viruses. In: Kuchler RJ, Ed. Biochemical Methods in Cell Culture and Virology. Stroudsburg, PA: Dowder, Hutchinson and Ross, Inc., pp117–167, 1977.
22. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg **27**:493–497, 1938.
23. Sanford BA, Thomas VL, Ramsay MA, Jones TO. Characterization of clinical strains of *Staphylococcus aureus* associated with pneumonia. J Clin Microbiol **24**:131–136, 1986.
24. Sanford BA, Davison VE, Ramsay MA. *Staphylococcus aureus* adherence to influenza A virus-infected and control cell cultures: Evidence for multiple adhesins. Proc Soc Exp Biol Med **181**:104–111, 1986.
25. Tobita K, Sugiura A, Enomoto C, Furuyama M. Plaque assay and primary isolation of influenza A viruses in an established line of canine kidney cells (MDCK) in the presence of trypsin. Med Microbiol Immunol **162**:9–14, 1975.
26. Bibel DJ, Aly R, Shinefield HR, Maibach HI, Strauss WG. Importance of the keratinized epithelial cell in bacterial adherence. J Invest Dermatol **79**:250–253, 1982.
27. Aly R, Shinefield HR, Litz C, Maibach HI. Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. J Infect Dis **141**:463–465, 1980.
28. Bibel DJ, Aly R, Shinefield HR, Maibach HI. The *Staphylococcus aureus* receptor for fibronectin. J Invest Dermatol **80**:494–496, 1983.
29. Freter R. Prospects for preventing the association of harmful bacterial with host mucosal surfaces. In: Beachey EH, Ed. Bacterial Adherence (Receptors and Recognition, Series B). New York: Chapman and Hall, Vol **6**: pp439–458, 1980.
30. Parsons CL, Mulholland SG. Bladder surface mucin. Its antibacterial effect against various bacterial species. Am J Pathol **93**:423–432, 1978.
31. Verghese A, Catanese A, Arbett RD. *Staphylococcus aureus* pneumonia in hamsters with elastase-induced emphysema—the virulence enhancing activity of mucin. Proc Soc Exp Biol Med **188**:1–6, 1988.
32. Vishwanath S, Ramphal R. Tracheobronchial mucin receptor for *Pseudomonas aeruginosa*: Predominance of amino sugars in binding sites. Infect Immun **48**:331–335, 1985.
33. Ishikawa H, Isayama Y. Evidence for sialyl glycoconjugates as receptors for *Bordetella bronchiseptica* on swine nasal mucosa. Infect Immun **55**:1607–1609, 1987.
34. Francis R Jr, Stuart-Harris CH. Studies on the nasal histology of epidemic influenza virus infection in the ferret I. The development and repair of the nasal lesion. J Exp Med **68**:789–802, 1938.
35. Liu C. Studies on influenza infection in ferrets by means of fluorescein-labelled antibody, I. The pathogenesis and diagnosis of the disease. J Exp Med **101**:665–676, 1955.
36. Sanford BA, Ramsay MA. Detection of staphylococcal membrane receptors on virus-infected cells by direct adhesin overlay. Infect Immun **52**:671–675, 1986.
37. Nugent KM, Pesanti EL. Tracheal function during influenza infections. Infect Immun **42**:1102–1108, 1983.
38. Plotkowski M-C, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of type I *Streptococcus pneumoniae* to tracheal epithelium of mice infected with influenza A/PR8 virus. Am Rev Respir Dis **134**:1040–1044, 1986.