Evidence of an Intracellular Reservoir in the Nasal Mucosa of Patients with Recurrent *Staphylococcus aureus* Rhinosinusitis

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Severe infections due to *Staphylococcus aureus* require prolonged therapy for cure, and relapse may occur even years after the first episode. Persistence of *S. aureus* may be explained, in part, by nasal carriage of *S. aureus*, which occurs in a large percentage of healthy humans and represents a major source of systemic infection. However, the persistence of internalized *S. aureus* within mucosal cells has not been evaluated in humans. Here, we provide the first in vivo evidence of intracellular reservoirs of *S. aureus* in humans, which were assessed in endonasal mucosa specimens from patients suffering from recurrent *S. aureus* rhinosinusitis due to unique, patient-specific bacterial clonotypes. Heavily infected foci of intracellular bacteria located in nasal epithelium, glandular, and myofibroblastic cells were revealed by inverted confocal laser scan fluorescence and electron microscopic examination of posttherapy intranasal biopsy specimens from symptom-free patients undergoing surgery on the sinuses. Intracellular residence may provide a sanctuary for pathogenic bacteria by protecting them from host defense mechanisms and antibiotic treatment during acute, recurrent *S. aureus* rhinosinusitis.

Staphylococcus aureus is a major pathogen that has a high potential for colonizing anterior nares or other body sites of both infected patients and healthy carriers [1–3]. Colonized patients are a predominant source of *S. aureus* in hospitals, and a substantial proportion of hospitalized patients develop severe *S. aureus* infections from their own endogenous nasal reservoirs [2–5]. Conflicting observations exist on the molecular mechanisms of *S. aureus* nasal colonization, which have been studied mostly in chronic carriers. Binding properties of *S. aureus* cell-surface components (e.g., cell-wall components such as teichoic acids) [2, 6] or cell wall–associated proteins,

known as "microbial surface components recognizing adhesive matrix molecules" [7, 8], with either carbohydrate-rich surface components of mucosal epithelial cells [2] or nasal mucus secretions [9], were extensively studied. Although it provides suitable explanation for initial binding and short-term carriage, extracellular binding of colonizing bacteria can hardly explain the long-term carriage of clonally identical strains of *S. aureus*, such as those found in a significant percentage of healthy carriers [4, 10–13]. Indeed, the presence of effective local defense mechanisms, such as the retrograde mucociliary transport, or locally secreted antimicrobial peptides [14, 15] should, in principle, lead to rapid elimination or replacement of colonizing organisms by exogenous strains [12, 16] and, thus, prevent long-term nasal carriage of identical strains.

Another mechanism that may potentially contribute to long-term endonasal persistence of *S. aureus* in some patients is the possibility of intracellular survival in a range of cell types frequently referred to as "nonprofessional phagocytes" [17, 18]. Notwithstanding its status as an extracellular pathogen [1], *S. aureus* reveals a considerable potential for in vitro invasion of epi-
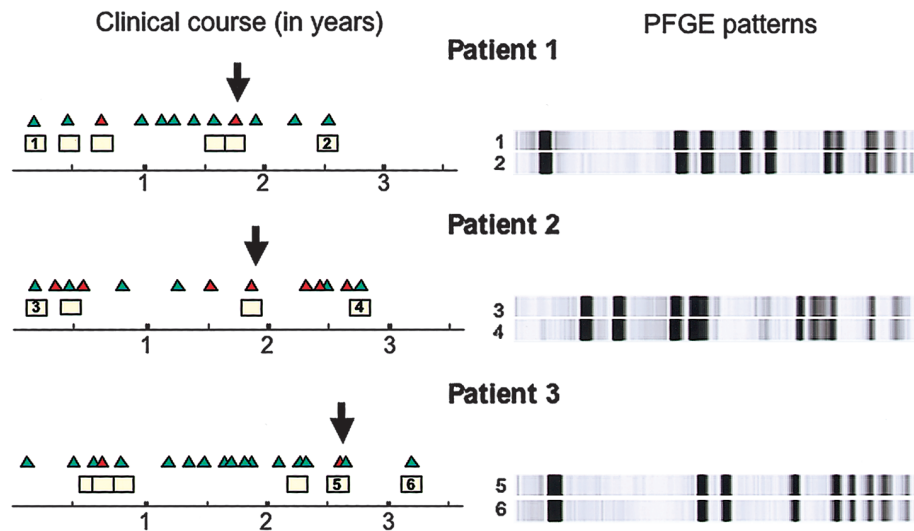
Figure 1. Clinical course for 3 patients during a 3-year period *(left panel)*. The occurrence of symptomatic episodes, either treated with systemic antibiotics or untreated, is indicated by green or red triangles, respectively. Black arrows indicate surgical biopsy specimens, and boxes represent *Staphylococcus aureus*–positive cultures. The nos. in boxes refer to strain isolates tested by pulsed-field gel electrophoresis (PFGE) *(right panel)*.

thelial and endothelial cells, osteoblasts, fibroblasts, and human embryonic kidney cell lines [18–28]. Several studies have demonstrated that fibronectin-binding proteins promote *S. aureus* internalization by binding to circulating or cellular fibronectin, which, in turn, acts as a molecular bridge to the host cell fibronectin receptor integrin $\alpha_5\beta_1$ [17–21, 23, 24, 26–29]. Integrin-mediated invasion of *S. aureus* into nonprofessional phagocytes is promoted by activation of Src family protein-tyrosine kinases [25, 28]. These in vitro data suggest that intracellular reservoirs might provide a sanctuary for *S. aureus* by protecting them from extracellular host defense mechanisms and from the most commonly used antibiotics, such as β-lactams, which do not penetrate into cells [17, 18]. However, evidence of such intracellular reservoirs and their in vivo contribution to persistence of *S. aureus* in humans is still lacking. The objective of the present study is to provide morphological evidence of intracellular foci of *S. aureus*, which may act as potential reservoirs for the multiple recurrent episodes of rhinosinusitis caused by unique, patient-specific clonotypes of this pathogen.

PATIENTS, MATERIALS, AND METHODS

Patients. This study was performed in compliance with the ethical guidelines established by the University Hospitals of Geneva. From January 2000 to December 2001, 750 outpatients were treated for persistent rhinosinusitis in the rhinology unit of our institution by endoscopic examination of the nasal cavities. The presence of polyps, crust, and/or purulent secretions in the middle meatus was observed in 135 patients (18%), of whom 39 also yielded *S. aureus*–positive cultures on samples from their middle meatus, conducted by endoscopy. Three of these patients required more-extensive investigations because

they experienced multiple rhinosinusitis episodes during a 3-year period (range, 12–18 episodes) and regularly relapsed with acute symptoms of nasal obstruction, anterior and posterior rhinorrhea, and headache within 2–3 months after standard systemic antimicrobial therapy and daily topical administration of nasal steroids (400 µg of mometasoni-17furoas) [30]. During each acute episode, patients' nasal mucosa, which was evaluated by endoscopic examination of the middle meatus, displayed an erythematous and edematous epithelium and the presence of polyps with purulent secretions (patients 1 and 3). The diagnosis of rhinosinusitis was established by direct coronal computed tomographic scanning, which showed inflammatory mucosa thickening ≥5 mm in >2 paranasal sinuses [31]. Direct endoscopically guided sampling of patients' middle meatus revealed the recurrent presence (during consecutive rhinosinusitis episodes) of *S. aureus* but not of other upper respiratory pathogens, such as *Streptoccoccus pneumoniae* and *Haemophilus influenzae*. Patients yielding *S. aureus*–positive cultures in the middle meatus were treated by standard regimens of oral antistaphylococcal antibiotics, in accordance with the in vitro sensitivity data (none of the *S. aureus* isolates displayed resistance to methicillin), combined with topical administration of nasal steroids (400 µg of mometasoni-17furoas), as recommended elsewhere [30]. At 2 weeks after completion of treatment, the overall therapeutic response of each patient was assessed for relief of symptoms, reduction in size of polyps, and disappearance of crusts and/or purulent discharge, as evaluated by endoscopic examination of the nasal cavities.

Endoscopic nasal surgery—including partial middle turbinectomy, middle meatotomy, and anterior ethmoidectomy—was performed with the patients under general anesthesia. Tissue
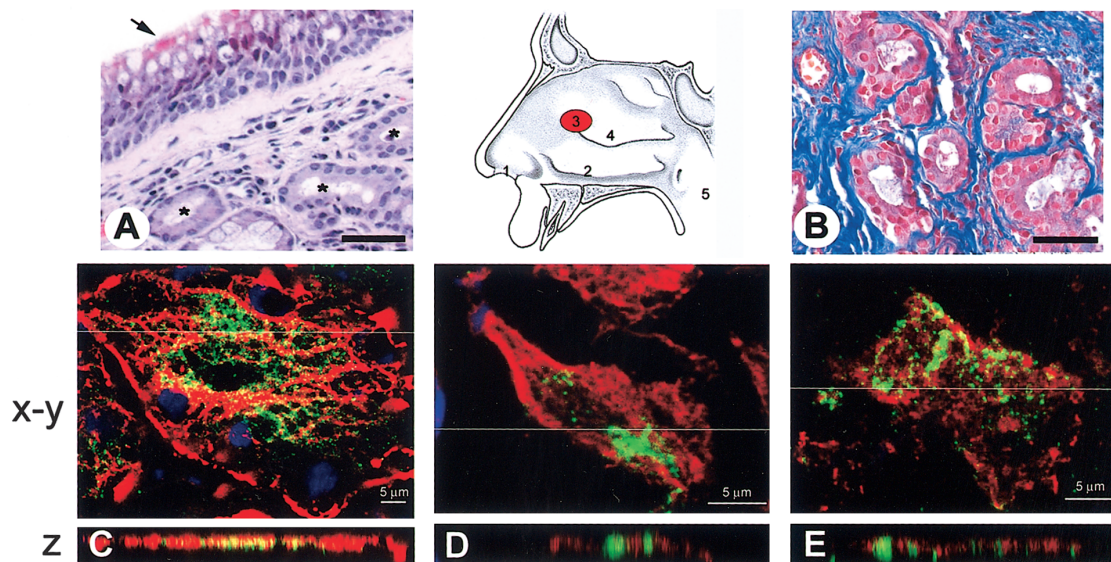
Figure 2. Histological location and immunohistochemical detection of *Staphylococcus aureus (center)*. Lateral view of the nasal wall, showing (1) anterior nares, (2) inferior turbinate, (3) anterior part of the middle turbinate (where surgical biopsy specimens were obtained) *(red)*, (4) middle turbinate, and (5) rhinopharynx. *A* and *B*, Standard histological views of dermal epithelium *(arrow)* and mucosal glands *(star)*. Sections were stained with hematoxylin-eosin *(A)*. Mucosal glands are surrounded by fibrotic tissue visualized by blue-stained collagen deposition *(B)* on Masson-trichrome–stained sections. Bar, 20 μm. *C–E*, Intracellular localization of *S. aureus* in various cell types, by confocal microscopy. *S. aureus* were labeled with specific antibody *(green)*, and cell nuclei were labeled with TOTO-III *(blue)*. Epithelial cells were visualized with anti-keratin *(red; C)*, myofibroblasts were visualized with anti–α–smooth muscle actin *(D)*, and inflammatory cells were visualized with anti-CD45 *(red; E)* antibodies. In the top images of each panel, projections were constructed from confocal Z stacks (0.5 μm thick). The bottom images are vertical views in the *Z*-plane and were obtained by combining the series of *X–Y* scans taken along the *Z*-axis. Lines drawn in the top images indicate where the *Z*-sections were generated.

specimens obtained during the surgical procedure were evaluated by routine anatomic, pathological, and histological procedures and were screened for the presence of microbial pathogens.

Bacteriological identification and molecular typing. Swabs of nasal secretions or biopsy specimens (disrupted in a sterile tissue grinder in 500 μL of 0.9% NaCl) were plated on blood, chocolate, and Columbia colistin nalidixic agar (Becton Dickinson), and then *S. aureus* was identified according to standard bacteriological criteria. Molecular typing of sequential *S. aureus* isolates was performed by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digested with *Sma*I (Bio-Rad), by use of a CHEF MAPPER system (Bio-Rad) [32]. DNA banding patterns of the different gels were analyzed by use of the software GelCompar (version 4.1; Applied Math) and interpreted according to criteria published elsewhere [32].

Indirect immunofluorescence, confocal laser scanning microscopy, and light microscopy. Nasal biopsy specimens were embedded in OCT 4583 (Miles Scientific) and frozen in precooled liquid isopentane. Three-micrometer cryostat sections were fixed in acetone for 5 min at $-20°C$ and stained with anti–*S. aureus* antibodies (mouse IgM; Chemicon International) combined with antibodies directed against either human keratin (wide-spectrum screen rabbit polyclonal antibody [Dako]) or α–smooth muscle actin mouse IgG2a [33] or human CD45 (mouse IgG1 [BD Biosciences PharMingen]). Control experiments as-

sessed the specificity of anti–*S. aureus* antibodies directed against staphylococcal peptidoglycan, which, under the conditions used for immunofluorescence, did not cross-react with *Staphylococcus epidermidis* or other gram-positive species. Subsequently, samples were incubated with Alexa 488–labeled anti-mouse IgM antibodies (Molecular Probes) and with secondary tetramethylrhodamine isothiocyanate–conjugated antibodies recognizing either rabbit (Jackson ImmunoResearch Labs) or the different mouse isotypes (Southern Biotechnology Associates); the secondary antibodies were diluted in PBS solution containing 1:100 human serum and 1:1000 nuclear dye (TOTO-III; Molecular Probes) and were mounted in polyvinyl alcohol mounting medium. An inverted confocal laser scan fluorescence microscope (Carl Zeiss) was used to collect 3-D image sets. Stacks of confocal images were processed by use of IMARIS software (version 3.2; Bitplane AG) for 3-D view of the infected cells. Simultaneous detection, by confocal imaging, of immunostained *S. aureus* and cytoplasmic components (e.g., cytokeratin) on 0.5-μm-thick cross-sectional images was performed to assess the intracytoplasmic localization of bacteria.

For light microscopic examination, pieces of tissue were fixed in 10% neutral buffered formol and embedded in paraffin. Four-micrometer serial sections were stained with either hematoxylin-eosin or blue alanine Masson's trichrome or were immunostained with anti–*S. aureus* antibodies. Immunoperoxidase staining was
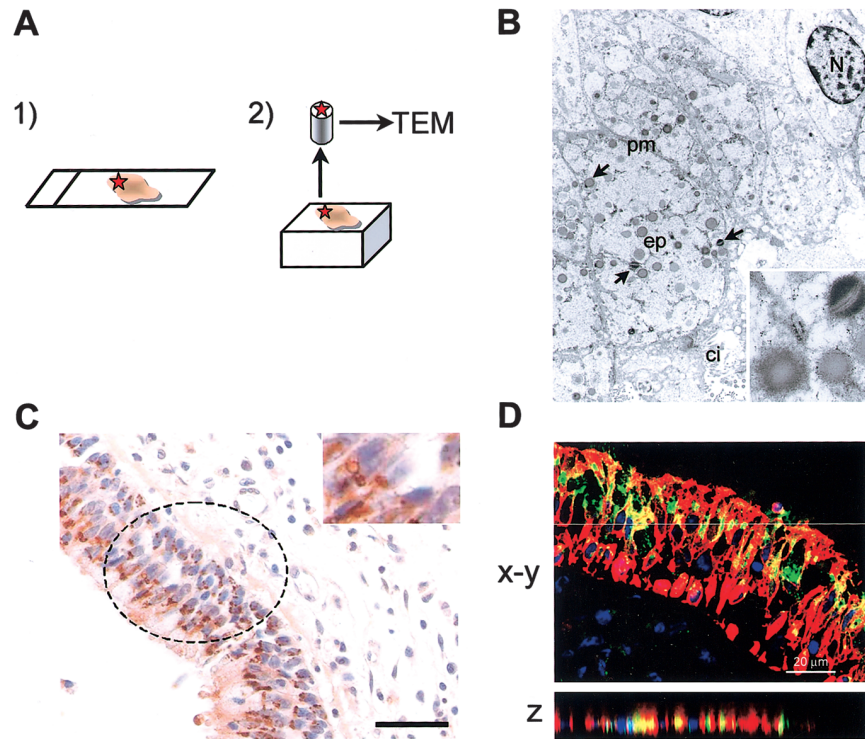
Figure 3. Presence of *Staphylococcus aureus* in epithelial cells of nasal epithelium, evaluated by transmission electron microscopy (TEM) *(arrows; B)*, immunohistochemical methods *(brown; C)*, and immununofluorescence *(S. aureus* in *green,* keratin in *red,* and nuclei in *blue; D). A,* Schematic representation of the multistep protocol used to optimize the detection of *S. aureus* by TEM. The highly infected areas *(star* corresponding to the *dashed circle* in *C)* were selected by microscopy (step 1), and fragments were punched from the paraffin-embedded block (step 2) and processed for TEM. Insets in *B* and *C* represent a zoomed portion of each image. ci, cilia from the cell apical surface; ep, epithelial cell; N, nucleus; pm, plasma membrane. Bars in *C* and *D,* 20 μm. Original magnification in *B,* $\times$2200; original magnification in *inset,* $\times$23,000.

performed as described elsewhere [34]. Sections were examined by use of an Axiophot photomicroscope (Carl Zeiss), and images were acquired by use of an Axiocam camera (Carl Zeiss).

Transmission electron microscopy (TEM). Highly infected areas were selected by microscopic examination of paraffin-embedded tissues stained, by immunochemical methods, with anti–*S. aureus* antibodies, as described above, and punch fragments of the paraffin-embedded block were then deparaffined in xylol and ethanol, followed by fixation in 1% osmium tetroxide for 1 h and subsequent dehydration and embedding in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined by use of a CM10 electron microscope (Philips).

RESULTS

The time course of multiple symptomatic rhinosinusitis episodes (range during a 3-year period, 12–18) for each of the 3 patients is shown in parallel with *S. aureus*–positive cultures of middle meatus samples and courses of systemic antimicrobial therapy combined with daily topical administration of nasal steroids (figure 1). PFGE data were used to assess the presence of single, patient-specific clonotypes of *S. aureus* during multiple rhinosinusitis episodes (figure 1).

Histological analysis of posttherapy intranasal biopsy specimens from patients with *S. aureus* rhinosinusitis undergoing surgery on the sinuses showed the presence of diffuse fibrotic areas surrounding mucous-producing glands (figure 2B) that were characterized by expression of α-smooth muscle actin (data not shown), an actin isoform typical of myofibroblastic cells, which are abundant and persistent in fibrotic inflammatory tissue [35]. Although the presence of *S. aureus* was easily detected by microbiological cultures of biopsy specimens, its detection by use of a cell wall–specific monoclonal antibody was far more difficult, because of highly irregular spatial distribution. Intracellular location of *S. aureus* was assessed by confocal immunofluorescence microscopic examination of nasal specimens that combined immunodetection of *S. aureus* with specific markers of different cell types. *S. aureus* frequently colocalized with cytokeratin (intracytoplasmic epithelia–specific intermediate filament protein) [36] in mucous glands (figure 2C) and nasal epithelial cells (figure 3D). By use of Z-scan, *S. aureus* clusters were also observed in myofibroblastic cells at the same level as was α-smooth muscle actin, which appeared to form a constricting ring around bacteria (figure 2D). As expected, intracellular *S. aureus* were also identified in CD45-

positive professional phagocytic cells distributed throughout the connective tissue, in epithelium, or in fibrotic areas (figure 2E). Confocal microscopic analyses provided further evidence that approximately one-third of the *S. aureus*–infected cells, regardless of the cell type, carried >10 bacteria.

Intracellular *S. aureus* were also seen by TEM examination of highly infected foci (selected by microscopic examination of tissue sections stained, by immunochemical methods, with anti–*S. aureus*) (figure 3C) that had been punched from paraffin-embedded tissue and reprocessed for TEM (figure 3A). This protocol was required for accurate localization of *S. aureus*–containing areas that were dispersed within nasal mucosa specimens. TEM examination (figure 3B) confirmed the presence of numerous intracytoplasmic *S. aureus* in nasal epithelial cells.

DISCUSSION

The mechanisms of persistence and recurrence of *S. aureus* in humans are still partly speculative. The lack of in vivo morphological evidence of intracellular *S. aureus* reservoirs, which may contribute to clinically important *S. aureus*–persistent diseases, might be explained, in part, by technical difficulties in localizing the highly focalized *S. aureus* intracellular reservoirs [17]. Despite its in vitro potential for invading a range of non-professional phagocytes [18–28], *S. aureus* has no dedicated molecular system of penetration, in contrast to enteroinvasive bacteria [37].

The fate of internalized *S. aureus* after endocytosis in cell-culture models may be determined by the interplay between host cell defense mechanisms and the intrinsic virulence of internalized *S. aureus* strains. A frequently described scenario is that of initial intraphagosomal survival, followed by *S. aureus* escape into the cytoplasm, followed by intracellular replication and eventually leading to host cell apoptosis [18, 38–44]. However, in vitro studies showing rapid (within 24–48 h) triggering of apoptosis by intracellular *S. aureus* are of uncertain relevance for explaining long-term persistence of bacteria in vivo. In this context, an important determinant shown to significantly improve *S. aureus* intracellular survival is the emergence or selection by the intracellular milieu of small colony variants (SCVs) displaying a decreased growth rate and diminished hemolytic activity [45, 46]. Several observations suggest that, by alteration of their metabolic properties and reduction of their virulence, SCVs may have increased intracellular survival as a result of prevention of or substantially delayed host cell lysis or apoptosis [45, 46]. Furthermore, SCVs also have been shown to have decreased susceptibility to some bactericidal antibiotics [47].

Demonstration of highly focalized intracellular reservoirs of *S. aureus* in various cell types of the endonasal mucosa from patients facing multiple episodes of recurrent rhinosinusitis represents an important step toward a more complete understanding of persistent infection. This observation raises several questions: (1) What are the risk factors contributing to the emergence of intracellular reservoirs of *S. aureus*? (2) If the intracellular residence of *S. aureus* indeed provides a sanctuary for the bacteria by protecting them from host defense mechanisms and commonly used antibiotic treatments, therefore explaining persistence and recurrence of *S. aureus* in these patients, how should antimicrobial therapy be optimized? (3) Does intracellular infection play a role in other severe *S. aureus* infections, such as endocarditis or osteomyelitis? (4) How can our study contribute to understanding of *S. aureus* colonization in asymptomatic patients, which is an extremely frequent event, affecting ~20%–30% of healthy individuals? Although patients with recurrent and severe *S. aureus* rhinosinusitis are more suitable for morphological studies—because they should have locally higher bacterial densities, compared with healthy carriers—several characteristics of *S. aureus* colonization look similar in both groups, such as recurrence or persistence of the same *S. aureus* clonotype for prolonged periods, even after attempts of decolonization with topical or systemic agents. This finding, however, remains speculative, and further studies are being undertaken to address these questions.

References

1. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med **1998**; 339: 520–32.
2. Kluytmans JA, Van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev **1997**; 10:505–20.
3. Muto CA, Jernigan JA, Ostrowsky BE, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. Infect Control Hosp Epidemiol **2003**; 24:362–86.
4. Von Eiff C, Becker K, Machka K, Stammer H, Peters G, Study G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med **2001**; 344:11–6.
5. Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet **2004**; 364:703–5.
6. Weidenmaier C, Kokai-Kun JF, Kristian SA, et al. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med **2004**; 10:243–5.
7. Patti JM, Allen BL, McGavin MJ, Höök M. MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol **1994**; 48:585–617.
8. O'Brien LM, Walsh EJ, Massey RC, Peacock SJ, Foster TJ. *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell Microbiol **2002**; 4:759–70.
9. Shuter J, Hatcher VB, Lowy FD. *Staphylococcus aureus* binding to human nasal mucin. Infect Immun **1996**; 64:310–8.
10. Goerke C, Papenberg S, Dasbach S, et al. Increased frequency of ge-

nomic alterations in *Staphylococcus aureus* during chronic infection is in part due to phage mobilization. J Infect Dis **2004**;189:724–34.

11. Cespedes C, Said-Salim B, Miller M, et al. The clonality of *Staphylococcus aureus* nasal carriage. J Infect Dis **2005**;191:444–52.

12. Peacock SJ, Justice A, Griffiths D, et al. Determinants of acquisition and carriage of *Staphylococcus aureus* in infancy. J Clin Microbiol **2003**; 41:5718–25.

13. Van Belkum A, Eriksen NHR, Sijmons M, et al. Coagulase and protein A polymorphisms do not contribute to persistence of nasal colonisation by *Staphylococcus aureus*. J Med Microbiol **1997**; 46:222–32.

14. Cole AM, Dewan P, Ganz T. Innate antimicrobial activity of nasal secretions. Infect Immun **1999**;67:3267–75.

15. Cole AM, Tahk S, Oren A, et al. Determinants of *Staphylococcus aureus* nasal carriage. Clin Diagn Lab Immunol **2001**;8:1064–9.

16. Nouwen J, Boelens H, Van Belkum A, Verbrugh H. Human factor in *Staphylococcus aureus* nasal carriage. Infect Immun **2004**;72:6685–8.

17. Lowy FD. Is *Staphylococcus aureus* an intracellular pathogen? Trends Microbiol **2000**; 8:341–3.

18. Alexander EH, Hudson MC. Factors influencing the internalization of *Staphylococcus aureus* and impacts on the course of infections in humans. Appl Microbiol Biotechnol **2001**; 56:361–6.

19. Dziewanowska K, Patti JM, Deobald CF, Bayles KW, Trumble WR, Bohach GA. Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. Infect Immun **1999**;67:4673–8.

20. Dziewanowska K, Carson AR, Patti JM, Deobald CF, Bayles KW, Bohach GA. Staphylococcal fibronectin binding protein interacts with heat shock protein 60 and integrins: role in internalization by epithelial cells. Infect Immun **2000**;68:6321–8.

21. Lammers A, Nuijten PJM, Smith HE. The fibronectin binding proteins of *Staphylococcus aureus* are required for adhesion to and invasion of bovine mammary gland cells. FEMS Microbiol Lett **1999**;180:103–9.

22. Jevon M, Guo C, Ma B, et al. Mechanisms of internalization of *Staphylococcus aureus* by cultured human osteoblasts. Infect Immun **1999**; 67:2677–81.

23. Massey RC, Kantzanou MN, Fowler T, et al. Fibronectin-binding protein A of *Staphylococcus aureus* has multiple, substituting, binding regions that mediate adherence to fibronectin and invasion of endothelial cells. Cell Microbiol **2001**;3:839–51.

24. Ahmed S, Meghji S, Williams RJ, Henderson B, Brock JH, Nair SP. *Staphylococcus aureus* fibronectin binding proteins are essential for internalization by osteoblasts but do not account for differences in intracellular levels of bacteria. Infect Immun **2001**;69:2872–7.

25. Fowler T, Johansson S, Wary KK, Hook M. Src kinase has a central role in in vitro cellular internalization of *Staphylococcus aureus*. Cell Microbiol **2003**;5:417–26.

26. Sinha B, Francois PP, Nüsse O, et al. Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. Cell Microbiol **1999**;1:101–18.

27. Nair SP, Bischoff M, Senn MM, Berger-Bächi B. The sigma B regulon influences internalization of *Staphylococcus aureus* by osteoblasts. Infect Immun **2003**;71:4167–70.

28. Agerer F, Michel A, Ohlsen K, Hauck CR. Integrin-mediated invasion of *Staphylococcus aureus* into human cells requires Src family protein-tyrosine kinases. J Biol Chem **2003**;278:42524–31.

29. Sinha B, Francois P, Que YA, et al. Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells. Infect Immun **2000**;68:6871–8.

30. Benninger MS, Hadley JA, Osguthorpe JD, et al. Techniques of intranasal steroid use. Otolaryngol Head Neck Surg **2004**;130:5–24.

31. Lund VJ, Kennedy DW. Staging for rhinosinusitis. Otolaryngol Head Neck Surg **1997**;117:S35–S40.

32. Blanc DS, Struelens MJ, Deplano A, et al. Epidemiological validation of pulsed-field gel electrophoresis patterns for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol **2001**;39:3442–5.

33. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol **1986**;103:2787–96.

34. Clement S, Chaponnier C, Gabbiani G. A subpopulation of cardiomyocytes expressing alpha-skeletal actin is identified by a specific polyclonal antibody. Circ Res **1999**; 85:e51–8.

35. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. J Pathol **2003**; 200:500–3.

36. Franke WW, Grund C, Osborn M, Weber K. The intermediate-sized filaments in rat kangaroo PtK2 cells. I. Morphology in situ. Cytobiologie **1978**;17:365–91.

37. Cossart P, Sansonetti PJ. Bacterial invasion: the paradigms of enteroinvasive pathogens. Science **2004**;304:242–8.

38. Bayles KW, Wesson CA, Liou LE, Fox LK, Bohach GA, Trumble WR. Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. Infect Immun **1998**;66:336–42.

39. Kahl BC, Goulian M, Van Wamel W, et al. *Staphylococcus aureus* RN6390 replicates and induces apoptosis in a pulmonary epithelial cell line. Infect Immun **2000**;68:5385–92.

40. Qazi SNA, Counil E, Morrissey J, et al. *agr* expression precedes escape of internalized *Staphylococcus aureus* from the host endosome. Infect Immun **2001**;69:7074–82.

41. Shompole S, Henon KT, Liou LE, Dziewanowska K, Bohach GA, Bayles KW. Biphasic intracellular expression of *Staphylococcus aureus* virulence factors and evidence for Agr-mediated diffusion sensing. Mol Microbiol **2003**;49:919–27.

42. Menzies BE, Kourteva I. *Staphylococcus aureus* α-toxin induces apoptosis in endothelial cells. FEMS Immunol Med Microbiol **2000**; 29: 39–45.

43. Esen M, Schreiner B, Jendrossek V, et al. Mechanisms of *Staphylococcus aureus* induced apoptosis of human endothelial cells. Apoptosis **2001**;6: 431–9.

44. Bantel H, Sinha B, Domschke W, Peters G, Schulze-Osthoff K, Janicke RU. Alpha-toxin is a mediator of *Staphylococcus aureus*-induced cell death and activates caspases via the intrinsic death pathway independently of death receptor signaling. J Cell Biol **2001**; 155:637–48.

45. Proctor RA, Peters G. Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. Clin Infect Dis **1998**; 27:419–22.

46. Vesga O, Groeschel MC, Otten MF, Brar DW, Vann JM, Proctor RA. *Staphylococcus aureus* small colony variants are induced by the endothelial cell intracellular milieu. J Infect Dis **1996**; 173:739–42.

47. Proctor RA, Kahl B, Von Eiff C, Vaudaux P, Lew DP, Peters G. Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin Infect Dis **1998**; 27(Suppl 1):S68–74.