Central role of a bacterial two-component gene regulatory system of previously unknown function in pathogen persistence in human saliva

Samuel A. Shelburne III*, Paul Sumby^{†‡}, Izabela Sitkiewicz^{†‡}, Chanel Granville[†], Frank R. DeLeo[§], and James M. Musser^{†‡¶}

Departments of *Medicine and [†]Pathology, Center for Human Bacterial Pathogenesis, Baylor College of Medicine, Houston, TX 77030; [‡]Center for Molecular and Translational Human Infectious Diseases Research, Methodist Hospital Research Institute, and Department of Pathology, Methodist Hospital, Houston, TX 77030; and [§]Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840

Edited by Richard M. Krause, National Institutes of Health, Bethesda, MD, and approved September 12, 2005 (received for review July 12, 2005)

The molecular genetic mechanisms used by bacteria to persist in humans are poorly understood. Group A *Streptococcus* (GAS) causes the majority of bacterial pharyngitis cases in humans and is prone to persistently inhabit the upper respiratory tract. To gain information about how GAS survives in and infects the oropharynx, we analyzed the transcriptome of a serotype M1 strain grown in saliva. The dynamic pattern of changes in transcripts of genes [*spy0874/0875*, herein named *sptR* and *sptS* (*sptR/S*), for saliva persistence] encoding a two-component gene regulatory system of unknown function suggested that *SptR/S* contributed to persistence of GAS in saliva. Consistent with this idea, an isogenic nonpolar mutant strain (Δ *sptR*) was dramatically less able to survive in saliva compared with the parental strain. Iterative expression microarray analysis of bacteria grown in saliva revealed that transcripts of several known and putative GAS virulence factor genes were decreased significantly in the Δ *sptR* mutant strain. Compared with the parental strain, the isogenic mutant strain also had altered transcripts of multiple genes encoding proteins involved in complex carbohydrate acquisition and utilization pathways. Western immunoblot analysis and real-time PCR analysis of GAS in throat swabs taken from humans with pharyngitis confirmed the findings. We conclude that *SptR/S* optimizes persistence of GAS in human saliva, apparently by strategically influencing metabolic pathways and virulence factor production. The discovery of a genetic program that significantly increased persistence of a major human pathogen in saliva enhances understanding of how bacteria survive in the host and suggests new therapeutic strategies.

gene expression | group A *Streptococcus* | microarray | *Streptococcus pyogenes*

The ability of microorganisms to alter gene expression in specific host environments is essential for survival and pathogenesis. Many human pathogens persist for prolonged periods in particular host niches (1, 2). Delineation of the molecular underpinnings of bacterial persistence in humans is critical to developing therapeutic agents that inhibit genes or gene products contributing to pathogen survival, thereby interrupting infection.

Group A *Streptococcus* (GAS) has a predilection for inhabiting the human oropharynx. The organism causes most cases of bacterial pharyngitis and colonizes up to one-half of all school-age children in nonepidemic periods (3). Importantly, GAS can persist in the human oropharynx for prolonged periods, even after treatment with appropriate antimicrobial agents (4). Although GAS has been known to be the major cause of bacterial pharyngitis for >80 years, we have only a rudimentary understanding of the molecular mechanisms used by this pathogen to survive in the human oropharynx.

We recently initiated study of GAS–human saliva interaction to gain insight into GAS activity during the earliest stages of upper respiratory tract infection (5). Saliva is ubiquitous in the human oropharynx and contains diverse molecules critical to innate and acquired immunity (6). Productive infection of the oropharynx and subsequent transmission of GAS to a new host requires pathogen survival and proliferation in saliva (7, 8). Many components of the innate and acquired immune systems present in saliva also are found at other mucosal surfaces where bacteria are common, including the lower respiratory, gastrointestinal, and female urogenital tracts (9–11). Thus, knowledge gained about how GAS responds to saliva may very well contribute to a broader understanding of host–pathogen interaction and microbial persistence on mucosal surfaces.

We recently discovered that multiple GAS strains unexpectedly persisted for 1 mo at very high cell densities ($\approx 10^7$ colony-forming units per ml) when grown *in vitro* in human saliva (5). Here we used iterative expression microarray analysis, immunologic methods, and *in vivo* gene quantification to identify a genetic program used by GAS to survive in saliva. A key discovery was that a two-component regulatory system (TCS) of unknown function played a central role in pathogen survival in saliva. Our findings reveal an intimate link between metabolism, virulence factor production, and bacterial persistence in saliva and suggest new therapeutic strategies.

Materials and Methods

Bacterial Strains. Strain MGAS5005 is genetically representative of the serotype M1 clone responsible for most contemporary (after 1987) human infections; its genome was recently sequenced (12, 13). The Δ *sptR* isogenic mutant strain was created from the parental serotype M1 strain MGAS5005 by nonpolar insertional mutagenesis (14).

Growth of GAS in Human Saliva. Saliva was collected from healthy adult volunteers under a protocol approved by the Baylor College of Medicine Institutional Review Board (5). The same

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: GAS, Group A *Streptococcus*; THY, Todd–Hewitt broth with yeast extract; *spy*, *Streptococcus pyogenes*; *Sic*, streptococcal inhibitor of complement; *SpeB*, streptococcal pyrogenic exotoxin B; TCS, two-component gene regulatory system; *SptR/S*, saliva persistence.

Data deposition: Expression microarray data have been deposited in the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo).

[¶]To whom correspondence should be sent at the present address: Center for Molecular and Translational Human Infectious Disease Research, Methodist Hospital Research Institute, and Department of Pathology, Methodist Hospital, 6565 Fannin Street, F816, Houston, TX 77030. E-mail: jmmusser@tmh.tmc.edu.

© 2005 by The National Academy of Sciences of the USA

Fig. 1. Growth curve and principal component analysis of expression microarray data for serotype M1 strain MGAS5005 in human saliva. (A) Growth of strain MGAS5005 in human saliva. GAS was grown in nutrient rich broth (THY) to $OD_{600} = 0.5$, then diluted 1:50 into human saliva. After 4 h of growth, a second 1:50 dilution was made into fresh human saliva (5), and growth was assayed by removing 100- μ l aliquots every 90 min for the first 9 h and then every 24 h. The aliquots were diluted in PBS, plated onto blood sheep agar, and incubated overnight, and colonies were counted. Arrows indicate times at which RNA was isolated for transcriptome analysis (4 and 16 h). Error bars represent standard deviation. (B) Shown is a principal components analysis plot, which captures the variance in a dataset in terms of principal components and displays the two most significant of these on the x and y axes. Red dots, 4-h time point; blue dots, 16-h time point; PC, principal component. Ellipses represent two standard deviations.

group of saliva donors was used for each portion of the studies described herein. To minimize potential variation generated by differences in donor saliva, the same pool of saliva was used for each experimental replicate performed at a particular phase of the research. For further details about growth of GAS in human saliva see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

Expression Microarray Analysis. RNA was isolated from five replicates with a Fast Prep Blue Kit (Qbiogene, Irvine, CA) (15) and purified by using an RNeasy kit (Qiagen, Valencia, CA). The concentration and quality of RNA were assessed with an Agilent Technologies (Palo Alto, CA) 2100 Bioanalyzer and analysis of the A_{260}/A_{280} ratio. An Affymetrix GeneChip was used for expression microarray studies (15).

Western Immunoblot Analysis. Proteins present in the culture supernatant of GAS strains were analyzed by Western immunoblot (5).

Analysis of Gene Expression in Human Pharyngitis Using TaqMan Real-Time RT-PCR Analysis. Throat swabs were taken from six patients presenting with acute GAS pharyngitis under a protocol approved by the Baylor College of Medicine Institutional Review Board. RNA was isolated and TaqMan real-time RT-PCR was performed with eight replicates for each sample (16).

Results and Discussion

Principal Component Analysis of Expression Microarray Data. Growth of serotype M1 strain MGAS5005 in human saliva consisted of an exponential phase followed by a prolonged stationary phase (Fig. 1A). Transcriptome analysis was performed on bacteria harvested after 4 h (exponential phase) and 16 h (stationary phase) of growth in saliva. Principal component analysis indicated that the data were of high quality and the two time points provided distinct information regarding GAS gene transcripts (Fig. 1B).

Transcriptome of GAS Early During Interaction with Human Saliva. GAS is transmitted from person to person mainly by organisms present in saliva (7, 8). To simulate GAS transmission, we grew bacteria in human saliva for several hours and transferred the organisms to fresh saliva (see *Materials and Methods*). Early (4 h) in growth in saliva, we found very high transcripts of genes encoding proteins that participate in host–pathogen interaction such as *emm* (*spy2018*; *spy*, *Streptococcus pyogenes*) encoding M protein and *sic* (*spy2016*) encoding streptococcal inhibitor of complement (Sic) (17) (Fig. 2A). M protein has been reported to bind salivary mucin, and Sic can inactivate antimicrobial peptides in saliva (19, 20).

Saliva imposes significant oxidative stress on invading microorganisms (21). Numerous GAS genes encoding proteins involved in the response to oxidative stress, such as *ahpC* (*spy2079*) encoding a putative peroxiredoxin reductase and *mtsA* (*spy0453*) encoding a metal-binding protein, had very high transcripts at both time points (Fig. 2B). At 4 h, we observed very high transcripts of genes in the arginine deiminase operon (*spy1541–8*) (Fig. 2C; see also Table 1, which is published as supporting information on the PNAS web site). Inasmuch as these genes encode proteins involved in amino acid metabolism, ATP production, and pH regulation (22), the results suggest that the high transcripts are a response to the limited nutrient supply and the relatively low pH of saliva (6). Taken together, the data show that saliva triggers changes in GAS transcripts presumably needed to survive and proliferate in this fluid.

GAS Gene Transcripts During Stationary Phase in Saliva. The limited nutrients present in human saliva may contribute to host defense by restricting the growth of microorganisms (6). We recently reported (5) that despite the lack of readily available nutrients, GAS maintained high numbers of colony-forming units in saliva for at least 28 days. We sought to gain new knowledge about the molecular mechanisms contributing to the unexpected ability of GAS to persist in human saliva for extended periods by analyzing transcripts at the stationary phase of growth. Eight of the 10 genes with the most significant increase in transcripts between 4 and 16 h encode proteins involved in the metabolism of carbon sources (Fig. 2C and Table 1). These included genes encoding starch degrading proteins AmyA (*spy1302*) and MalX (*spy1306*), phosphotransferase enzymes AgaD (*spy0629*) and MalE (*spy1059*), and carbohydrate metabolism enzymes (*spy1399*). Similarly, 7 of the top 10 genes with the greatest decrease in transcripts between the 4- and 16-h time points also encoded proteins involved in carbohydrate acquisition or metabolism, including *pulA* (*spy1972*), encoding a pullulanase, and MalE (*spy1294*), encoding an inferred maltose-binding protein (Fig. 2C and Table 1).

We also identified marked changes in transcripts for virulence factors between the two time points (Fig. 2A and Table 1). *spy1915* (*salA*) encoding salivaricin A, a secreted lantibiotic that inhibits growth of many oropharyngeal microorganisms (23) was highly expressed at 4 h relative to 16 h. Conversely, at the 16 h time point there was significantly increased expression of *spd* (*spy2043*) and *spd3* (*spy1436*) encoding secreted DNases involved

Fig. 2. Analysis of gene transcripts in serotype M1 strain MGAS5005 during growth in human saliva. Aliquots were removed for RNA isolation after growth in human saliva for 4 (red bars) and 16 h (blue bars). Data shown are $\log_2$ -fold expression normalized to *proS* transcript levels. The values are averages obtained from five independent samples. Shown are data for genes encoding proven and putative virulence factors (A), proven and putative oxidative stress response proteins (B), proven and putative amino acid and carbohydrate metabolism proteins (C), and proven and putative transcriptional regulators (D). *spy* (*S. pyogenes*) gene numbers are assigned to serotype M1 GAS strain SF370 ORFs (18). Error bars represent standard deviation.

in immune evasion, dissemination, and perhaps nutrient utilization (24); *speB* encoding streptococcal pyrogenic exotoxin B (SpeB), *sagA* (encoding streptolysin S, a cytolytic toxin), and *spy0470*, encoding a 67-kDa myosin crossreactive protein (Fig. 2A). Taken together, these data show that GAS significantly remodels its transcriptome during growth in saliva, the result being enhanced competition, nutrient acquisition, and survival.

Transcripts of GAS Transcriptional Regulators During Growth in Human Saliva. The observed alterations in GAS gene transcripts at the two time points suggested that changes also occurred in the expression of genes encoding regulatory proteins. For example, transcript levels of *rofA* (*spy0124*), which encodes a transcriptional regulator important for GAS adhesion to eukaryotic cells, were significantly higher at the 4- compared with the 16-h time point (25) (Fig. 2D). Conversely, at the 16-h time point, there were markedly increased transcripts of *perR* (*spy0187*), *spy0583*, and *hrcA* (*spy1763*), three genes encoding putative or proven stress response transcriptional regulators (26). High transcript levels of the *ihk-irr* TCS were also present at the 16-h time point (Fig. 2D). Inasmuch as *Ihk-Irr* is known to be critical to the ability of GAS to survive oxidative stress (27), *Ihk-Irr* may be at least partially responsible for the high transcripts of various

oxidative stress response genes noted above. Taken together, the dynamic changes in transcripts of many known and putative transcriptional regulators indicated that GAS utilizes multiple complex regulatory networks during its interaction with human saliva.

Saliva and the *spy0874/0875* TCS of Unknown Function. The genome of strain MGAS5005 contains 13 TCS, including 12 that are highly conserved among GAS strains (12). Although the functions of several of these TCS have been described (27–29), the great majority remain of unknown function. As a first step toward investigating whether one or more of the TCS is crucial to the successful interaction of GAS with saliva, we examined the expression microarray data for all TCS at 4 and 16 h. *spy0874/0875* had the highest transcripts at the 16-h time point and the greatest increase in transcripts between the 4- and 16-h time points (Fig. 2D and Table 1).

Δ *spy0874* Isogenic Mutant Strain and Growth Characteristics in Human Saliva. The results suggested that *spy0874/0875* contributed to persistence of GAS in human saliva. To test this hypothesis, we created a Δ *spy0874* isogenic mutant strain from wild-type strain MGAS5005 by nonpolar insertional mutagen-

Fig. 5. Western immunoblot analysis of actively secreted GAS virulence factors in wild-type (serotype M1 strain MGAS5005) or its Δ sptR isogenic mutant derivative. GAS was grown in human saliva or standard laboratory media (THY) for the indicated time periods. Supernatants were adjusted to be derived from 5×10^8 colony-forming unit GAS, and proteins were precipitated with 30% trichloroacetic acid and separated by using 12% SDS/PAGE. Proteins were transferred to a membrane and probed with primary and secondary antibodies as described in *Materials and Methods*. SpeB, streptococcal pyrogenic exotoxin B; Mac, streptococcal Mac protein (also known as IdeS); Sic, streptococcal inhibitor of complement. Note that Western immunoblot for SpeB at 4 h of growth in saliva or early exponential phase in THY is not shown, because no protein production was observed at that time point, consistent with the observation that SpeB is not produced until the early stationary phase.

oropharynx (14, 24, 30). Importantly, along with *sic*, *speB* was recently shown to be key to the successful persistence of GAS in human saliva (5) and was also up-regulated in the wild-type strain. Taken together, analysis of the transcriptome of the Δ sptR isogenic mutant strain suggested a broad deficiency in the ability of the mutant strain to adequately express genes encoding crucial determinants of GAS oropharyngeal colonization and infection.

Marked Reduction in the Transcripts of Genes Encoding Multiple Known and Putative Carbohydrate Utilization Proteins in the Δ sptR Isogenic Mutant Strain. Nutrient limitation is thought to be a major contributor to GAS growth restriction in human saliva (5, 6). As noted above, for wild-type GAS, many of the genes with the highest transcripts at the 16-h time point encoded known or putative carbohydrate utilization proteins. We hypothesized that the inability of the Δ sptR isogenic mutant strain to persist in human saliva was linked to a deficiency in optimal metabolism of complex carbohydrates. At both time points, we observed marked differences in the transcripts of a wide variety of genes encoding known and putative carbohydrate utilization proteins, a result consistent with this hypothesis (Figs. 4B and 8 and Table 2). Genes encoding putative carbohydrate utilization proteins with increased transcripts in the wild-type strain included *amyA* (*spy1302*) (cyclodextrin glucanotransferase), *malE* (*spy1294*) (maltose-binding protein), *araD* (*spy0179*) (ribose epimerase), and *asaD* (*spy0629*) (phosphotransferase system enzyme). Importantly, we did not observe significant transcript differences in genes encoding proteins putatively dedicated to the transport and utilization of simple sugars such as glucose (*spy1986*). Taken together, these data strongly suggest that one of the major roles of the SptR/S TCS is to coordinate expression of genes involved in acquisition and processing of complex carbohydrates.

Western Immunoblot Analysis of Secreted GAS Virulence Factors. We used Western immunoblot analysis to test the hypothesis that the decreased transcripts of genes encoding secreted virulence factors observed in the Δ sptR isogenic mutant strain resulted in decreased exoprotein production during growth in saliva. Consistent with the transcriptome data, no substantial differences were detected between the wild-type and mutant strains in the

Fig. 6. Transcripts of *sptR* are present *in vivo* during pharyngitis in humans. Transcript levels for *sptR* were determined by TaqMan real-time PCR in six patients with GAS pharyngitis. Circles show the M serotype of the infecting GAS strain. Median transcript values are in parentheses below M type and are expressed relative to the GAS *proS* gene transcripts (31). Error bars represent standard deviation.

amount of immunoreactive SpeB, Sic, or streptococcal Mac protein present in human saliva at 4 h (Fig. 5). However, at 16 h, the amount of immunoreactive material present in the saliva supernatant of the parental serotype M1 strain MGAS5005 was much higher compared with the supernatant of the mutant strain for these three proteins (Fig. 5). To judge whether the observed effects were specific for growth in saliva, we also analyzed supernatants obtained during growth in THY. Importantly, at no time point did we observe differences in the amount of immu-

Fig. 7. Hypothesis explaining how the SptR/S TCS contributes (directly or indirectly) to persistence of GAS in human saliva. After recognition of presently unidentified signals present in human saliva, SptS undergoes autophosphorylation and then phosphorylates SptR, allowing for interaction with DNA. Phosphorylation of SptR results in changes in a broad range of proteins putatively involved in the metabolism of complex carbohydrates and may directly affect the production and/or secretion of numerous GAS virulence factors. Alternatively, the metabolic changes induced by SptR may act directly or indirectly through other regulatory pathways to affect virulence factor production. The relationship of the protein names to the cell surface corresponds with their inferred location (e.g., actively secreted, anchored to the cell wall, embedded in the cell membrane, or in the cytosol).

noreactive material present for any of three proteins when the two strains were grown in THY. Also of note, no immunoreactive SpeB was detected in supernatants obtained from GAS grown in THY, whereas in striking contrast, abundant immunoreactive SpeB was present after 16 h of growth in saliva. Inasmuch as SpeB is a major contributor to the persistence of GAS in human saliva (5), these results show that GAS responds to growth in saliva by secreting a protein necessary for survival. Taken together, these data indicate that SptR/S affects production of at least three secreted GAS virulence factors during growth in human saliva, thereby confirming the gene expression data. Importantly, these differences were not observed for GAS grown in THY, stressing the key role of SptR/S in GAS-saliva interaction.

Expression of *sptR* in Human Pharyngitis Patients. Given the crucial contribution of *sptR* to persistence of GAS in saliva *ex vivo*, we hypothesized that *sptR* was transcribed in humans with GAS pharyngitis. We tested this hypothesis by assessing *sptR* gene transcripts by using TaqMan real-time PCR and RNA isolated from throat swabs taken from six patients with GAS pharyngitis (31) (see Table 3, which is published as supporting information on the PNAS web site). Transcripts of *sptR* were present in all patient strains and were an average of 3-fold higher compared with the internal control gene *proS* (Fig. 6). Thus, *sptR* is highly transcribed in humans, strongly suggesting that SptR/S contributes to the pathogenesis of GAS pharyngitis.

Comment

Despite being recognized as the leading cause of bacterial pharyngitis in humans for >80 years, relatively little is known about the molecular mechanisms used by GAS to colonize and infect the human upper respiratory tract. Our analysis of GAS-human host-pathogen interaction by using *ex vivo* cultivation in human saliva generated an enhanced view of how GAS responds to the survival challenges faced in the human oropharynx. We found that GAS reacted to growth in human saliva by highly

transcribing genes encoding proteins involved in diverse physiological processes such as nutrient acquisition, response to oxidative stress, and evasion of innate and acquired immune system components. Our finding of high transcripts of diverse carbohydrate metabolism genes early during GAS interaction with human saliva correlates well with recently published GAS gene expression during experimental monkey pharyngitis (32).

A key discovery was our identification of the role played by the TCS *sptR/S* in successful long-term persistence of GAS in human saliva. Recent analysis (5) of 11 other TCS and stand-alone gene transcription regulators failed to identify a role in GAS saliva persistence, thereby stressing the unique nature of the *sptR/S* findings. The ability of bacteria to persist in saliva and other human environments is a central aspect of the life cycle of many pathogens. Despite extensive genome-wide analyses of GAS (33), before our study, there was little knowledge of the role played by *sptR/S* in host-pathogen interaction. Importantly, a recent study of GAS gene expression over 86 days in the monkey oropharynx noted that the transcript dynamics of *sptR/S* suggested a role for this TCS in GAS pathogenesis (32). Thus, two highly independent lines of investigation converged to identify SptR/S as a crucial contributor to host-pathogen interaction in the upper respiratory tract. We hypothesize that SptR/S links central metabolic processes with the production of a broad range of putative and known virulence factors (Fig. 7). If this is the case, then interrupting the function of this TCS may be therapeutically beneficial. Moreover, together with a recent study (5), our results provide insight into the molecular methods used by a major human bacterial pathogen to persistently inhabit a specific host milieu.

Microbial persistence in the host is fundamental to the natural history of many infectious agents. Our findings suggest that similarly structured genome-wide studies of other pathogens may be a fruitful line of investigation likely to yield an enhanced understanding of pathogenesis.

This work was supported in part by National Institutes of Health Grants UO1-A160595 (to J.M.M.) and T32-19503 and K12-RR17665 (to S.A.S.).

- Young, D., Hussell, T. & Dougan, G. (2002) *Nat. Immunol.* **3**, 1026–1032.
- Stewart, G. R., Robertson, B. D. & Young, D. B. (2003) *Nat. Rev. Microbiol.* **1**, 97–105.
- Peter, G. & Smith, A. L. (1977) *N. Engl. J. Med.* **297**, 365–370.
- Gerber, M. A., Tanz, R. R., Kabat, W., Bell, G. L., Siddiqui, B., Lerer, T. J., Lepow, M. L., Kaplan, E. L. & Shulman, S. T. (1999) *Pediatrics* **104**, 911–917.
- Shelburne, S. A., Granville, C. N., Tokuyama, M., Sitkiewicz, I., Patel, P. & Musser, J. M. (2005) *Infect. Immun.* **73**, 4723–4731.
- Amerongen, A. V. & Veerman, E. C. (2002) *Oral Dis.* **8**, 12–22.
- Hamburger, M. (1944) *J. Infect. Dis.* **75**, 71–78.
- Hamburger, M. & Robertson, O. (1948) *Am. J. Med.* **4**, 690–701.
- Iimura, M., Gallo, R. L., Hase, K., Miyamoto, Y., Eckmann, L. & Kagnoff, M. F. (2005) *J. Immunol.* **174**, 4901–4907.
- Bikker, F. J., Ligtenberg, A. J., van der Wal, J. E., van den Keijbus, P. A., Holmskov, U., Veerman, E. C. & Nieuw Amerongen, A. V. (2002) *J. Dent. Res.* **81**, 134–139.
- Prakobphol, A., Xu, F., Hoang, V. M., Larsson, T., Bergstrom, J., Johansson, I., Frangmyr, L., Holmskov, U., Leffler, H., Nilsson, C., et al. (2000) *J. Biol. Chem.* **275**, 39860–39866.
- Sumby, P., Porcella, S. F., Madrigal, A. G., Barbican, K. D., Virtaneva, K., Ricklefs, S. M., Sturdevant, D. E., Graham, M. R., Vuopio-Varkila, J., Hoe, N. P., et al. (2005) *J. Infect. Dis.* **192**, 771–782.
- Musser, J. M. & Krause, R. M. (1998) in *Emerging Infections*, ed. Krause, R. (Academic, New York), pp. 185–218.
- Lukowski, S., Hoe, N. P., Abdi, I., Rurangirwa, J., Kordari, P., Liu, M., Dou, S. J., Adams, G. G. & Musser, J. M. (2000) *Infect. Immun.* **68**, 535–542.
- Graham, M. R., Virtaneva, K., Porcella, S. F., Barry, W. T., Gowen, B. B., Johnson, C. R., Wright, F. A. & Musser, J. M. (2005) *Am. J. Pathol.* **166**, 455–465.
- Chaussee, M. S., Watson, R. O., Smoot, J. C. & Musser, J. M. (2001) *Infect. Immun.* **69**, 822–831.
- Cunningham, M. W. (2000) *Clin. Microbiol. Rev.* **13**, 470–511.
- Ferretti, J. J., McShan, W. M., Ajdic, D., Savic, D. J., Savic, G., Lyon, K., Primeaux, C., Sezate, S., Suvorov, A. N., Kenton, S., et al. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 4658–4663.
- Ryan, P. A., Pancholi, V. & Fischetti, V. A. (2001) *Infect. Immun.* **69**, 7402–7412.
- Frick, I. M., Akesson, P., Rasmussen, M., Schmidtchen, A. & Bjorck, L. (2003) *J. Biol. Chem.* **278**, 16561–16566.
- Battino, M., Ferreira, M. S., Gallardo, I., Newman, H. N. & Bullon, P. (2002) *J. Clin. Periodontol.* **29**, 189–194.
- Chaussee, M. S., Somerville, G. A., Reitzer, L. & Musser, J. M. (2003) *J. Bacteriol.* **185**, 6016–6024.
- Upton, M., Tagg, J. R., Wescombe, P. & Jenkinson, H. F. (2001) *J. Bacteriol.* **183**, 3931–3938.
- Sumby, P., Barbican, K. D., Gardner, D. J., Whitney, A. R., Welty, D. M., Long, R. D., Bailey, J. R., Parnell, M. J., Hoe, N. P., Adams, G. G., et al. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 1679–1684.
- Beckert, S., Kreikemeyer, B. & Podbielski, A. (2001) *Infect. Immun.* **69**, 534–537.
- Ricci, S., Janulczyk, R. & Bjorck, L. (2002) *Infect. Immun.* **70**, 4968–4976.
- Voyich, J. M., Sturdevant, D. E., Braughton, K. R., Kobayashi, S. D., Lei, B., Virtaneva, K., Dorward, D. W., Musser, J. M. & DeLeo, F. R. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 1996–2001.
- Graham, M. R., Smoot, L. M., Migliaccio, C. A., Virtaneva, K., Sturdevant, D. E., Porcella, S. F., Federle, M. J., Adams, G. J., Scott, J. R. & Musser, J. M. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 13855–13860.
- Kreikemeyer, B., Boyle, M. D., Buttar, B. A., Heinemann, M. & Podbielski, A. (2001) *Mol. Microbiol.* **39**, 392–406.
- Ashbaugh, C. D., Moser, T. J., Shearer, M. H., White, G. L., Kennedy, R. C. & Wessels, M. R. (2000) *Cell. Microbiol.* **2**, 283–292.
- Virtaneva, K., Graham, M. R., Porcella, S. F., Hoe, N. P., Su, H., Graviss, E. A., Gardner, T. J., Allison, J. E., Lemon, W. J., Bailey, et al. (2003) *Infect. Immun.* **71**, 2199–2207.
- Virtaneva, K., Porcella, S. F., Graham, M. R., Ireland, R. M., Johnson, C. A., Ricklefs, S. M., Babar, I., Parkins, L. D., Romero, R. A., Corn, G. J., et al. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 9014–9019.
- Musser, J. M. & DeLeo, F. R. (2005) *Am. J. Pathol.*, in press.