visceral adipocytes of transgenic mice release sufficient corticosterone into the portal vein to alter the levels exposed to the liver. Indeed, portal vein corticosterone levels in Tg mice were increased 2.7-fold [129 ± 11.1 ng/ml (non-Tg) compared with 349 ± 79.5 ng/ml (Tg), $P < 0.01$] (Fig. 2E). Thus, visceral fat may affect hepatic metabolism by portal production of glucocorticoids as well as FFA.

Glucocorticoids regulate adipose tissue differentiation, function, and distribution, and their systemic excess produces a syndrome of central obesity with diabetes, hyperlipidemia, and hypertension, known as Cushing's syndrome (*3–5*). Although subtle alterations in the endocrine hypothalamic pituitary adrenal (HPA) axis have been reported in some studies of obesity (*4*, *5*, *30*), these have been controversial, and no clear role for increased circulating glucocorticoids in visceral obesity has emerged. On the other hand, a role for increased local cortisol reactivation in human obesity is suggested by several findings (*5–7*, *31*). 11β HSD-1 activity is higher in human visceral compared with subcutaneous adipose tissue (*31*), and reactivation of cortisone to cortisol is increased selectively in adipose tissue of obese humans, while impaired in liver (*7*). Similar findings were reported in obese Zucker rats (*8*). The thiazolidinedione (TZD) class of antidiabetic agents that are ligands for peroxysome proliferator–activated receptor (PPAR) γ markedly reduce adipocyte 11β HSD-1 mRNA and activity both in vivo and in vitro (*32*). Because TZDs preferentially reduce visceral fat accumulation in humans (*3*, *5*, *22*, *33*), suppression of adipose 11β HSD-1 by TZDs could be a mechanism for this fat redistribution and may play a role in their antidiabetic effects.

Our finding that a modest increase in the activity of 11β HSD-1 in adipose tissue of mice is sufficient to cause hyperphagia with visceral obesity and its most critical metabolic complications demonstrates that glucocorticoid-dependent adipocyte pathways have an unexpectedly major impact on systemic biology. Adipose tissue of obese humans is reported to have increased activity of 11β HSD-1 of similar or greater magnitude than that observed in our transgenic mice (*7*). These findings strongly suggest that increased adipocyte 11β HSD-1 is a common molecular mechanism for visceral obesity and the metabolic syndrome and may be an exciting pharmaceutical target for the treatment of this prevalent disorder.

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36. Supported by grants from NIH (J.S.F.) and Eli Lilly (J.S.F.), a programme grant from the Wellcome Trust (J.R.S. and J.J.M.), and grants from the Transgenic Core Facility of the Boston Obesity Nutrition Research Center and Animal Physiology Core of the Diabetes Endocrinology Research Center. H.M. held research fellowships from Uehara Memorial Foundation, Welfide Research Foundation, and Yamanouchi Foundation for Research on Metabolic Disorders. We thank K. Araki, B. B. Kahn, J. K. Hamm, Y.-B. Kim, Y. Minokoshi, I. Shimomura, R. S. Ahima, N. Tsuboyama-Kasaoka, H. Fang, and B. R. Walker for their invaluable advice and L. Ungsunann, L. Ramage, J. Lawitts, M. Meikle, O. Peroni, E. Hadro, and R. Rossi for their excellent assistance.

17 September 2001; accepted 25 October 2001

Rapid Killing of *Streptococcus pneumoniae* with a Bacteriophage Cell Wall Hydrolase

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Nasopharyngeal carriage is the major reservoir for *Streptococcus pneumoniae* in the community. Although eliminating this reservoir would greatly reduce disease occurrence, no suitable intervention has been available for this purpose. We show here that seconds after contact, a purified pneumococcal bacteriophage lytic enzyme (Pal) is able to kill 15 common serotypes of pneumococci, including highly penicillin-resistant strains. In vivo, previously colonized mice revealed undetectable pneumococcal titers 5 hours after a single enzyme treatment. Pal enzyme had little or no effect on microorganisms normally found in the human oropharynx, and Pal-resistant pneumococci could not be detected after extensive exposure to the enzyme.

Streptococcus pneumoniae remains one of the most challenging human pathogens because of the morbidity and mortality it causes in young children, the elderly, and in immunocompromised patients. The asymptomatic carrier state, particularly in children, is thought to be the major reservoir of the pathogen. Pneumococci account for several million cases of acute otitis media and an estimated 60,000 cases of invasive disease in the United States each year, with a mortality of 10% (*1*). Because of the worldwide increase of resistance to multiple antibiotics in pneumococci, treatment has become more difficult than in the past.

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Prevention of pneumococcal disease relies today on vaccination of the susceptible population. However, vaccination encounters a number of problems such as the limited quantity of serotypes in the pediatric formulation, incomplete protection against colonization, and selection of nonvaccine serotypes (*2*, *3*). There is need for an alternative preventive strategy for situations where vaccination is insufficient, impossible, or inefficient. Eradication or even reduction of nasopharyngeal carriage is likely to have a major impact on the transmission of *S. pneumoniae* and the incidence of infection. Antibiotic prophylaxis in controlled surroundings has shown limited success but carries the risk of selective pressure resulting in an increase of resistant strains (*4*). Until now, there has been no substance that can specifically reduce the number of pneumococci carried on human mu-

cous membranes without affecting the normal indigenous mucosal flora.

Since its discovery, the destructive effect of bacteriophage on their host organisms has been exploited to develop strategies to kill infecting bacteria (*5, 6*). Recently, Nelson *et al.* developed a method in which the purified lytic enzyme from streptococcal phage C1 was able to kill group A streptococci in vitro and to prevent and eliminate pharyngeal colonization in mice (*7*). We continued this line of investigation and explored the lytic action of a pneumococcal phage enzyme and its potential to kill *S. pneumoniae*. All known pneumococcal phages contain a cell wall lytic system consisting of a holin that permeabilizes the cell membrane, and either an *N*-acetylmuramoyl-L-alanine amidase (amidase) or a lysozyme, capable of digesting the pneumococcal cell wall (*8*). Both types of enzymes contain a COOH-terminal choline-binding domain and an NH_2-terminal catalytic domain. The lytic system allows the virus to escape the host cell after successful replication. We obtained *Escherichia coli* DH5α (pMSP11) expressing the amidase Pal of phage Dp-1 (*9*) from R. Lopez. We produced the enzyme in *E. coli* and purified it by affinity chromatography in a single step as described, with some modifications (*10, 11*). We then defined a unit for the enzyme, using lysis of exponentially growing *S. pneumoniae* serogroup 14 with serial dilutions of purified Pal (*12*).

In the first series of experiments, we measured the killing ability of Pal in vitro by exposing 15 clinical strains of *S. pneumoniae*, 2 pneumococcal mutants (R36A, Lyt 4-4), and 8 species of oral commensal streptococci (*S. gordonii*, *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius, S. intermedius, S. crista,* and *S. parasanguis*) to purified enzyme at a final concentration of 100 U/ml, and in the case of the oral streptococci, to 1000 and 10,000 U/ml (*13*). The pneumococcal strains, obtained from various sources [Web table 1 (*11*)], included nine serogroups that most frequently cause invasive disease in North America, Europe, Africa, and Oceania (*14*). Furthermore, three highly penicillin-resistant strains were included, which represent the internationally spread clones Sp^9-3, Sp^{14}-3, and Sp^{23}-1, that account for a majority of penicillin-resistant pneumococci in daycare centers and hospitals (*15, 16*). Within 30 s, 100 U of Pal decreased the viable titer of the 15 strains of exponentially growing *S. pneumoniae* by $\log_{10}$ 4.0 colony-forming units (cfu)/ml (median, range 3.3 to 4.7) as compared to controls incubated with the enzyme buffer alone (Fig. 1). Pneumococci with intermediate (n = 1) and high penicillin resistance (n = 3) were killed at the same rate as penicillin-sensitive strains [median (range) $\log_{10}$ 4.0 (3.7 to 4.7) versus $\log_{10}$ 4.1 (3.3 to 4.7) cfu/ml, P = not significant]. Moreover, the capsule-deficient laboratory strain R36A and the mutant Lyt 4-4, deficient in capsule and lacking the major pneumococcal autolysin LytA, showed identical susceptibility to Pal as the clinical pneumococcal isolates (decrease of $\log_{10}$ 4.2 and 3.9 cfu/ml, respectively, P = not significant). The latter results suggest that the pneumococcal capsule does not interfere with the enzyme's access to the cell wall and that autolysin does not contribute significantly to cell lysis caused by Pal. One-hundred units of Pal also killed exponentially growing *S. oralis* and *S. mitis*, but at a significantly lower rate ($\log_{10}$ 0.8 and $\log_{10}$ 0.23 cfu/ml, respectively, $P < 0.05$). Both strains are known to incorporate choline in their cell walls and therefore provide a binding site for the enzyme (*17*). The remaining oral streptococcal strains were unaffected at enzyme concentrations as high as 10,000 U/ml and up to 10 min of exposure.

In vitro, *S. pneumoniae*, including the R36A and Lyt 4-4 mutants, in stationary phase were more resistant to the lethal action of Pal. Nevertheless, exposure to 10,000 U/ml resulted in killing of $\log_{10}$ 3.0 cfu/ml (median, range 3.0 to 4.0) in 30 s. The mechanism responsible for the decrease in susceptibility to hydrolysis by Pal in nongrowing pneumococci is likely to be a change in the cell wall structure (*18*), such as an increase in peptidoglycan cross-linking.

Electron microscopy of *S. pneumoniae* serogroup 14 exposed to only 50 U/ml of Pal for 1 min (*19*) revealed protrusions of the cell membrane and the cytoplasm through single breaks in the cell wall, which appeared predominantly near the septum of the dividing diplococci (Fig. 2B). After 5 min, empty cell walls remained, retaining their original shape, indicating that digestion of amide bonds in a restricted location within the cell wall is sufficient for cell death (Fig. 2D).

The ability of Pal to eradicate *S. pneumoniae* from a mucosal surface was then tested in vivo in a mouse model of nasopharyngeal colonization (*20*) with minor modifications (*21*). In the first experiment, 18 mice were colonized intranasally with 10^8 cfu of *S. pneumoniae* serogroup 14. Animals were randomized 42 hours later for nasal and pharyngeal treatment with a total of 1400 U of Pal enzyme or buffer. Five hours after the treatment, all animals were killed, and pneumococcal titers were analyzed in the nasal wash. Treatment with Pal reduced *S. pneumoniae* to undetectable levels ($\log_{10}$ 0 cfu/10 μl nasal wash) as opposed to treatment with buffer only [median (range) $\log_{10}$ 3.0 (2.0 to 3.0) cfu/10 μl, $P < 0.001$] (Fig. 3A). We repeated the experiment with a lower dose of enzyme, randomizing

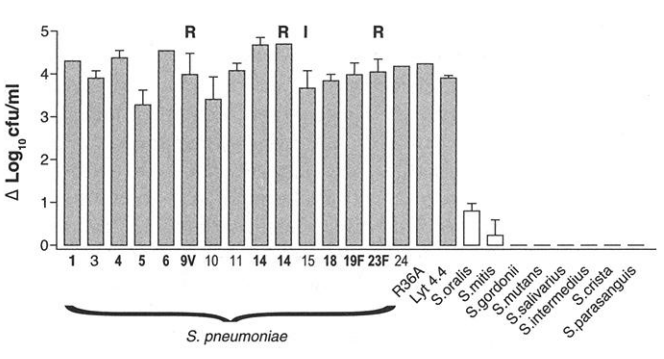

Fig. 1. In vitro killing of 15 clinical *S. pneumoniae* strains, 2 pneumococcal mutants, and 8 oral streptococcal species in log-phase with 100 U/ml Pal during 30 s, expressed as the decrease of bacterial titers in powers of 10. Numbers above "*S. pneumoniae*" indicate serogroups/serotypes; bold print designates the nine most frequently isolated serogroups. Error bars show standard deviation of triplicates. I, intermediate susceptibility to penicillin [minimum inhibitory concentration (MIC) = 0.1 to 1.0]; R, highly penicillin-resistant (MIC ≥ 2.0).

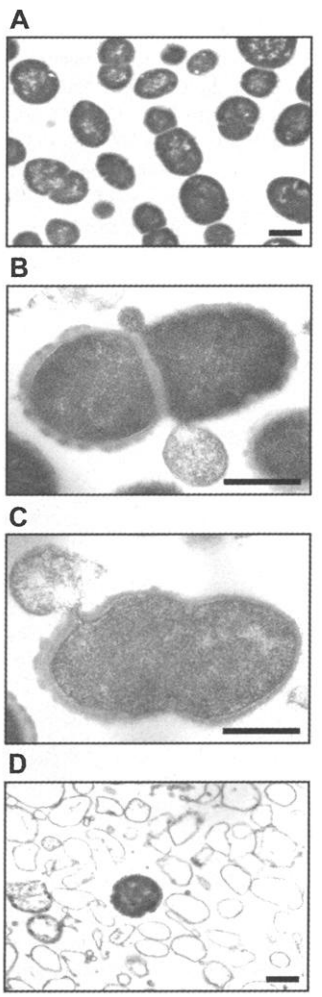

Fig. 2. Electron micrographs of *S. pneumoniae* exposed to 50 U of Pal. (**A**) shows unexposed control cells. Higher magnification of enzyme-treated cells after 1 min exposure shows cell membrane protrusion (**B**) or cytoplasmic leaks from membrane rupture (**C**) through isolated breaks in the cell wall. After 5 min (**D**), killing is virtually complete and only empty cell walls are left [magnification as in (A)]. Bars, 0.5 μm.

the animals ($n = 16$) for treatment with 700 U of Pal or buffer. Enzyme treatment here completely eliminated pneumococci from five of eight animals and significantly decreased titers in the remaining three ($P < 0.001$) (Fig. 3B). Each experiment included three uncolonized control animals that revealed no *S. pneumoniae*. These results indicate that pneumococci on mucosal surfaces are highly susceptible to the action of the lytic enzyme.

We further addressed the question whether surviving pneumococci are able to recolonize the nasopharynx of mice after a single dose of enzyme. Thirty mice were colonized as before (day −2) and randomized 42 hours later for treatment with 1400 U of Pal or buffer. Three mice of each group were killed on days 0, 2, 4, 6, and 8 and the nasal wash was analyzed. All buffer-treated mice remained colonized throughout the experiment (mean $\log_{10}$ 3.0) with titers decreasing progressively from day 6. Pal-treated mice had sterile nasal cultures on day 0 (5 hours after treatment), and thereafter, there was a discrete reappearance of pneumococci in one or two of the three animals tested, with titers significantly lower throughout day 6 and disappearance on day 8 [$P < 0.0001$ for the comparison of both curves, two-way analysis of variance (ANOVA)] [Web fig. 1 (*11*)]. Surviving pneumococci, therefore, were unable to reestablish titers sufficient for successful recolonization in mice.

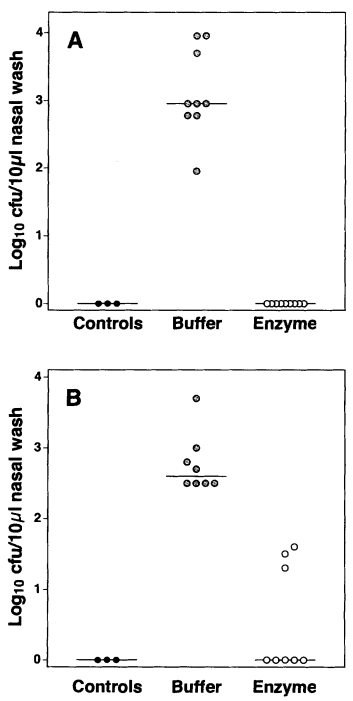

Fig. 3. Elimination of *S. pneumoniae* serotype 14 in the mouse model of nasopharyngeal carriage. **(A)** After nasal and pharyngeal treatment with a total of 1400 U of Pal, no pneumococci were retrieved in the nasal wash, compared to buffer-treated colonized mice ($P < 0.001$). No pneumococci were isolated from noncolonized control mice. **(B)** After treatment with a total of 700 U of Pal, pneumococci were completely eliminated in five of eight colonized mice ($P < 0.001$) and overall were significantly reduced. Bars show median.

Repeated exposure to low concentrations of Pal (<1 U) on agar plates or increasing concentrations in liquid assays did not lead to the recovery of resistant strains (*22*). This may be related to the fact that the cell wall receptor for Pal and other pneumococcal phage lytic enzymes is choline, a molecule that is necessary for pneumococcal viability (*9, 23, 24*). While not yet proven, it is possible that during a phage's association with bacteria over the millennia, to avoid being trapped inside the host, the binding domain of lytic enzymes has evolved to target a unique and essential molecule in the cell wall, making resistance to these enzymes a rare event.

We believe that with Pal and similar bacteriophage lytic enzymes, we may have the opportunity to control or eliminate nasopharyngeal colonization by *S. pneumoniae* and thus significantly reduce or prevent infection by these bacteria. Because bacteriophage have been described for nearly all bacteria, this targeted approach to control and/or prevent infection may be applied to other pathogens (particularly Gram-positive bacteria) whose reservoir or site of infection is the human mucous membrane.

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25. We especially thank R. Lopez who generously supplied his clone for the Pal enzyme; A. Tomasz and his team for all pneumococcal strains and for advice and encouragement. We also thank E. Sphicas for the preparation of the electron micrographs. Supported by a grant from the Defense Advance Research Projects Agency (to V.A.F.). J.M.L. was supported by fellowships from the Swiss National Science Foundation and the Frieda Locher-Hofmann Foundation.

4 October 2001; accepted 25 October 2001

Central Role of the CNGA4 Channel Subunit in Ca^{2+}-Calmodulin–Dependent Odor Adaptation

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Heteromultimeric cyclic nucleotide–gated (CNG) channels play a central role in the transduction of odorant signals and subsequent adaptation. The contributions of individual subunits to native channel function in olfactory receptor neurons remain unclear. Here, we show that the targeted deletion of the mouse *CNGA4* gene, which encodes a modulatory CNG subunit, results in a defect in odorant-dependent adaptation. Channels in excised membrane patches from the *CNGA4* null mouse exhibited slower Ca^{2+}-calmodulin-mediated channel desensitization. Thus, the CNGA4 subunit accelerates the Ca^{2+}-mediated negative feedback in olfactory signaling and allows rapid adaptation in this sensory system.

Olfactory receptor neurons (ORNs) respond to odorant stimulation with a receptor-mediated increase in intracellular cyclic adenosine 3′,5′-monophosphate (cAMP), which directly activates a cyclic nucleotide–gated (CNG) channel in the plasma membrane (*1*). Calci-